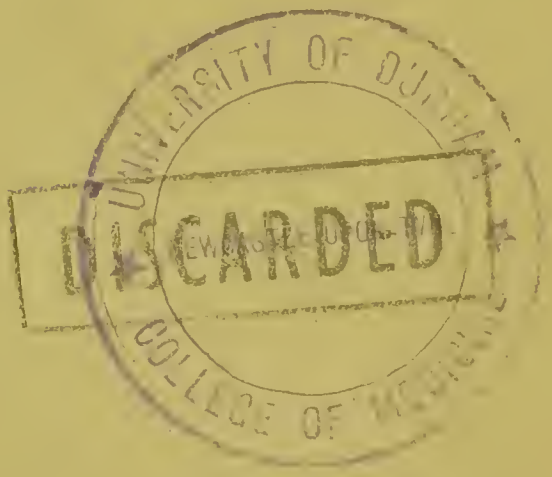

PRACTICAL

HISTOLOGY AND PATHOLOGY

BY

HENEAGE GIBBES, M.B.

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H. K. LEWIS, 136 GOWER STREET, W.C.
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PREFACE.

The object of this small work is to lay before the practitioner and student of medicine, a few concise and simple methods, by which the various tissues of the body may be prepared for examination with the microscope.

I do not claim any originality in these methods, but I recommend them from my own personal experience as the best, easiest, and cheapest to carry out.

The use of dyes for staining the tissues is a comparatively new branch of the science of Histology, and its value is being proved every day.

I have been engaged in experiments with all the various colouring agents for a long time, and in trying their effect in double and treble staining, and I have given the result of those which have proved most successful hitherto. I have also added a list of the aniline dyes, those soluble in water, and those soluble in spirit, which will be found very useful by anyone wishing to make experiments in staining.

Briefly, but sincerely, I offer my best thanks to Dr. Klein, F.R.S., for the assistance which he has at all times freely and generously given me.

H. G.

94 Gower Street.
October 1, 1880.

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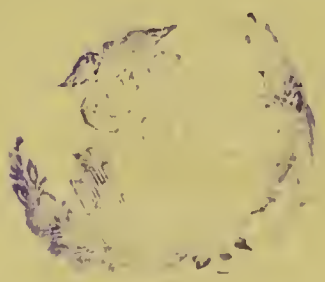

PRACTICAL HISTOLOGY AND PATHOLOGY.

CHAPTER I.

Introduction.

Histology or the minute anatomy of healthy tissue as revealed by the microscope, has made vast strides in the last few years, and now forms a most important part of Medical education; a thorough knowledge of the normal structure of the animal tissues being absolutely necessary for the appreciation of pathological change.

This subject is now taught at the Medical schools, and there is no excuse for a student who has finished his curriculum without some knowledge of practical histology, and of the minute anatomy of the human body.

There are many, however, who qualified some years ago when little attention was paid to this subject, who have consequently no practical knowledge of the methods required to prepare any morbid growth they may meet in their professional career, or how to set about a microscopical examination of the same.

With a view to help those who have not been able to get the necessary knowledge during their student career, and also those students who wish to form a Laboratory at home, the present work has been written,

giving only the ordinary methods used by the author in his own Laboratory.

When all these methods have been thoroughly worked out, the student will find himself competent to try any of the various processes mentioned in larger works, and to judge of their utility.

Many men on reading the different hardening, cutting, staining, and mounting processes which any tissue has to undergo before it can be examined with the microscope, will be inclined to think it very tedious work. It is, however, a mere matter of routine, and when once this routine is established, the whole thing is comparatively simple. It takes very little time to change the hardening fluid, and if the student gets into the habit of looking over the bottles on the shelf every morning, where he keeps those tissues in the process of hardening, a glance at the labels will show those requiring a change. When the sections are mounted and examined under the microscope, he will find himself amply repaid for all his trouble if he has faithfully carried out the different processes in every detail.

It is always better to have one or two shelves devoted to those preparations which require changing; and those such as chromic acid, which require fresh fluid often, should be kept by themselves. Each bottle should be labelled, and the tissue, date, and hardening fluid, clearly written on the label. Every morning this shelf should be examined, and those requiring it, changed; the date being each time written on the label, so that it may be seen at a glance how long the tissue has been in the fluid, and whether the hardening

agent ought to be renewed. Müller's fluid, and bichromate of potash preparations, may be placed by themselves, and need only be looked at occasionally.

A large outlay is not required for a course of Histological investigation, and the following list will show all those articles, re-agents, &c., which the student will find absolutely necessary.

1. Microscope. 1 Eye-piece. 2 Object glasses.
2. ½ gross ground edge, 3+1 slides.
3. 1 oz. No. 1, ¾ square cover glasses.
4. 1 oz. No. ⅞ ,, ,, ,,
5. 1 hollow ground razor.
6. Needles in handles.
7. 2 pair sharp pointed forceps.
8. 1 pair broad pointed forceps, not roughed, for taking up clean cover glasses.
9. Copper lifter.

Re-agents.

¾ per cent. Salt solution.
½ ,, Solution Nitrate of Silver.
½ ,, ,, Chloride of Gold.
5 ,, ,, Bicarbonate of Soda.

Glycerine
Canada Balsam } In drop bottles.
Dammar Varnish

A Bottle of Hollis' Glue.
A Williams' Microtome.
Watch Glasses.
Glass Capsules.
Dissecting Case.
Curved Scissors.

The Microscope.

The most expensive as well as the most important article required is the microscope, and a good one should be procured.

In purchasing a microscope, it is necessary to go to an optician who makes his own instruments to get one that is worth anything, as a large number are made by wholesale manufacturers and sold to various traders who put their names on them and sell them as their own make. It is an easy matter to find out a *bona fide* maker, as there are very few of them, and it is their interest to sell a good instrument. A microscope such as that required by anyone beginning a course of histology, can be procured for £5 5*s*., and nothing worth having can be bought at a lower price. If the instrument be procured from a *bona fide* maker it will last a lifetime with ordinary care.

The compound microscope consists of the stand, eye-piece, and object glasses. The stand should be a tripod having a stage of blackened glass, and a draw-tube lined with cloth. The tripod foot gives perfect steadiness, and for this reason the Continental models with horse-shoe foot have been given up by many of the best microscopists.

The price of these stands, with two object glasses, ranges from £5 5*s*. to £6 12*s*. 6*d*.

The student should ascertain by looking down the tube with the eye-piece removed, whether the hole in the stage is concentric with the tube, and then try the different holes in the diaphragm in the same way. The

diaphragm is an important part of the stand, as it is very often necessary to cut off some of the rays of light to get the best effect with high powers; and for this purpose the tubular diaphragm which is slipped into a fitting under the stage, is the best; it has a very small hole, and can be removed altogether when using a low power; a good plan is to have no fixed diaphragm at all, but a small piece of metal with a millimetre hole in the centre, made so that it can be slipped into the hole in the stage from above, where it will lay flush with the glass plate. It must be carefully centred with the tube.

Having selected a maker, the student should get him to explain the working of the different parts, as all that is necessary for a beginner can be learnt in ten minutes in that way.

This small student's stand is all that will be required by the student for a long time, as with it high powers can be used and whatever work may be done in the future, it will always be the working stand.

Good students' microscopes are made by Mr. Crouch, 66 Barbican; Messrs. Swift and Son, University Street.

The Object Glass. The object glass or objective, is the most important part of the microscope, and it is necessary to have good glasses to do satisfactory work.

The most useful for the student are the $\frac{2}{3}$ or $\frac{2}{5}$ for a low power, and the $\frac{1}{4}$, $\frac{1}{5}$, or $\frac{1}{6}$ for a high power; with a $\frac{2}{3}$ and $\frac{1}{6}$ the student can do all the requisite work for a long time, and then with the addition of a $\frac{1}{8}$, and oil immersion $\frac{1}{12}$, he would be set up for life.

The student should get some good microscopist to test the object glasses for him before purchasing them,

and he should see that they are tested on some histological object, and not on diatoms, as the wide angle necessary for resolving test diatomaceæ are the reverse of useful to the young Histologist. The most important glass for the student is the high power, and it is necessary that this should be a good one.

High Powers. The best high powers are made by Messrs. Powell and Lealand, they are of course expensive but pay well in the end; they are made with correction collars and are more adapted to large stands with mechanical stage. Zeiss' E = $\frac{1}{8}$ and F = $\frac{1}{12}$ are very good glasses, and well adapted to the students' stand and are not expensive; they have no correction collar, and the cover glasses should be measured to get the best effect; ·006 will do very well for D and E, and ·004 for F, the same applies to Mr. Crouch's $\frac{1}{6}$ which is corrected to a cover glass of ·006.

Oil Immersion Lenses. These glasses are taking the place of water immersion high powers in Histological research, as they have no correction for thickness of cover glass, and are consequently much easier to use, the only drawback is that the essential oil used will dissolve Canada balsam, Dammar varnish, and many of the other sealing fluids, and it is necessary to cover them with Hollis' glue which is not acted on by cedar oil.

These glasses were first made by Zeiss of Jena, and since by Messrs. Powell and Lealand. The first glass made by Zeiss was the $\frac{1}{8}$, he then brought out a $\frac{1}{12}$ and afterwards a $\frac{1}{18}$, of these the $\frac{1}{12}$ is unquestionably the best glass. Messrs. Powell and Lealand have made $\frac{1}{10}$, $\frac{1}{12}$, $\frac{1}{16}$, and $\frac{1}{25}$ on this principle, and have succeeded in removing a great objection to oil immersion lenses;

they have made their glasses perfectly homogeneous, that is, they require no change of the oil when using oblique light on an object, and no correction with the draw tube is necessary when using a very thin cover. With Zeiss' $\frac{1}{12}$ it is necessary to draw out the tube two inches with a ·004 cover glass, and for central light a mixture of fennel and olive oils is required, which must be changed to cedar oil for oblique light.

With Messrs. Powell and Lealand's glasses cedar oil is used indifferently for central or oblique light, and no correction is necessary for a thick or thin cover glass.

In using oil immersions, all that is required is to place a very small drop of oil on the front lens, screw the glass into the body, and lower it on to the slide until contact is made, which can be seen by bringing the eye to a level with the surface of the slide. The glass is then focussed by the fine adjustment until the object is seen sharply defined. With these glasses the best effect is obtained the moment the glass is in focus, and they have an enormous superiority over a dry glass of the same power in sharpness of definition and brilliancy. All slides intended for use with high powers should be sealed with Hollis' glue, and then the cedar oil left on the cover glass can be wiped off without the least danger to the preparation.

Eye-Pieces. Students' microscopes are generally sold with two eye-pieces, nos. 1 and 3, or A and C, but the no. 1 or A is the only one required, as no good result is obtained by using an eye-piece with a higher magnifying power, the higher eye-piece only magnifying the image seen with the object glass.

Higher eye-pieces are, however, useful in testing an object glass, as very many of those sold, while giving fairly good results with a no. 1 eye-piece, become blurred and indistinct when a no. 3 or 4 is used.

Illumination. Daylight is the best light to use for Histological work, and in the summer time there is generally enough light for ordinary work. In the winter, however, a lamp is often required even in the daytime, as there are many days when sufficient light cannot be obtained.

It is necessary, therefore, to be provided with a lamp burning either gas or parafine oil. The best gas lamp is made by Baker, it is a modification of the Highley microscopic lamp and is fitted with a Sugg's burner, it will be found very useful. An ordinary parafine lamp which can be purchased for 2s., with a $\frac{3}{4}$ inch flat wick, will be found all that is necessary by those who do not wish to get the more expensive gas lamp. It has also this advantage, the wick being flat the edge of the flame can be turned to the microscope, giving an intensity of light very useful in the investigation of fine structures under very high powers.

Achromatic Condenser.

A small condenser made to go into the fitting under the stage will be found very useful, but for high power work with a large stand a wide angled condenser is necessary, having a series of stops for oblique light, and a graduated diaphragm by which the rays of light can be gradually cut off until the best definition is obtained.

Stand Condenser.

It is better to use the direct light of the lamp without the intervention of a stand condenser, as by this instrument the light is diffused and the sharpness of definition impaired. A stand condenser is required to throw a light on opaque preparations, but as these are not often used by the student of Histology, it is not required by a beginner.

On Large Stands for High Powers.

The advanced student will require a Stand with mechanical stage and sub-stage when he makes investigations into minute structures with high powers, and for this work it is most important that the mechanism of the Stand should be of the very best description. To get this he must go to the best makers, and of course be prepared to pay a high price.

The best Stands are made by Messrs. Powell and Lealand, and Messrs. Ross and Co.

The author has used a No. 2 stand of Messrs. Powell and Lealand for a long time, and found it all that could be desired.

On the Binocular Microscope.

The binocular microscope has not met with much favor from Histologists as yet, from the fact that no power above a $\frac{1}{2}$ inch could be used without a special stand or apparatus costing a large sum. As, however, it has been shown by the author* that the binocular can be

* See *Quarterly Journal of Microscopical Science* for July, 1880.

used with the $\frac{1}{12}$ oil immersion, the student should try it, and see what a different view it gives of various structures and their relation to one another. Any ordinary stand made on the Jackson-Lister model will do, so that the body can be brought almost into contact with the stage. A Zeiss' D can be made to work perfectly well by fitting the front part into an adapter, so as to bring the lenses close to the prism. The relief to the eyes is very great.

For a higher power Messrs. Powell and Lealand make their $\frac{1}{12}$ oil immersion with a screw cut on the outside, so that the front part containing the lens can be screwed into an adapter, which they supply with the glass. By this means perfect stereoscopic effect is obtained, and the observer is enabled to realise the precise relations of the different structures he is looking at to one another.

CHAPTER II.

On Preparing Tissues for Examination.

The most essential point in microscopic investigation is the proper hardening of the material to be examined, and this must be done gradually, as if any tissue is placed in a strong solution, the elements of which it is composed at once shrink, and it is impossible to form any correct idea of their nature.

1. *Chromic Acid Mixture.* The most useful hardening agent is a mixture of chromic acid and spirit. Make a solution of chromic acid in water 15 grains to the pint, this is about $\frac{1}{6}$ per cent. Take of this 2 parts; methylated spirit 1 part.

The material must be cut into small pieces about half an inch square, and a large quantity of fluid used, a wide mouthed stoppered bottle holding from 6—10 ozs., according to the quantity of material, is best; change the fluid at the end of 24 hours, and again every third day, and the material will be hardened in from 8—12 days, this can be easily proved by taking out a piece and feeling it. If allowed to remain too long it gets brittle. When it is found to be moderately hard, usually after about 9—10 days, pour off the chromic acid mixture, and wash well, replace it by dilute spirit made thus:—

Take

Methylated Spirit 2 parts, and
Water 1 part.

Let the material remain in this for from 24—36 hours, never longer than three days, and then replace it by pure methylated spirit, it may remain in this for an indefinite time, but it will often be found that the spirit becomes cloudy and full of deposits in a few days; in this case it is only necessary to change the spirit until it remains clear.

A large quantity of $\frac{1}{6}$ per cent. solution of chromic acid should be kept on hand, and it should be mixed with the spirit as required, it will be found the most useful of all the hardening agents, if it is changed at the proper times, and it should not be used stronger than is given above, it will even be found beneficial to use it weaker in some cases.

2. *Müller's Fluid* is a good hardening mixture, but requires a much longer time, taking weeks to do what the chromic acid mixture will do in days. It is made thus:—

Take

Potass. Bichrom. 2 parts.
Sodæ Sulph. 1 part.
Water 100 parts.

The advantage of this mixture is that larger pieces can be hardened in it, and it does not require changing after the first week or two, but it will take from 5—7 weeks to harden anything according to its size. The material, when sufficiently hardened, should be well washed and then placed in dilute spirit in the same manner as recommended after hardening in the chromic acid mixture.

3. *Dilute Spirit.* Many tissues can be hardened in spirit alone if they are placed in dilute spirit at first, so that the elements of which they are composed are not shrunk. This process is also used after hardening by any of the others.

Dilute spirit is made by adding 1 part of water to 2 parts of methylated spirit.

The material to be hardened should not be left in this mixture more than from 24—48 hours.

It is then transferred to pure methylated spirit.

4. *Bichromate of Potash.* Make a 2 per cent. solution and keep it on hand, as it is very useful for many tissues that require slow hardening. A solution can be made much more quickly with warm water than cold. This solution is also very useful to place portions of morbid material in, on their removal from the body in the post-mortem room, they can afterwards be transferred to the chromic acid mixture for more rapid hardening. This solution takes from three to seven weeks to harden according to the size of the specimen, and the frequency with which the solution is changed.

5. *Bichromate of Ammonia.* A 2 per cent. solution is used in precisely the same manner as the former, and is applicable to the same tissues.

6. *Chromate of Ammonia.* Make a 5 per cent. solution, that is 1 oz. of the salt to 20 oz. of water, and filter. Keep it in a stoppered bottle. When this hardening agent is used for fresh tissue, such as mesentery, a small quantity is placed in a glass vessel and the tissue immersed in it for 24 hours, it is then washed until no more colour comes away and mounted in glycerine.

For other tissues it is necessary that the material should be cut into small pieces and left in the solution for 24—48 hours. It is then placed in distilled water, which must be changed several times until it is no longer tinged. The hardening is completed by the spirit process (page 13).

7. *Chloride of Gold.* Half per cent. solution. This is sold in small glass tubes, each containing 15 grains of the chloride, equal to 7 grains of pure gold. Take one of these tubes and file a ring round it above the bulb, it can then be easily divided into two parts, empty the gold chloride into a 6-ounce bottle, and wash out any particles that remain with distilled water, fill up the bottle. This will be under a half per cent. solution, but answers very well. Place a small quantity of this solution in a watch glass and immerse the tissue, which must be perfectly fresh, in it, let it remain in the dark for from half to one hour or more, then place in distilled water, which must be changed several times, and expose to diffuse daylight until it becomes a violet brown; about 24 hours will do in summer.

The tissue can then be mounted in glycerine, if it is a small thin substance, such as a Tadpole's tail.

If, however, larger portions of any tissue are stained with gold chloride, the hardening will not be sufficient, and they must be further hardened by the spirit process (page 13).

Mouse-tail, stained and hardened by the gold process, may be decalcified by placing it in a half per cent. solution of chromic acid or a saturated solution of picric acid for a few days.

For another gold process, see Cornea. Gold chloride has a staining as well as a hardening action.

8. *Picric Acid.* A saturated solution of picric acid will decalcify small bones. It is also used in some cases as a hardening agent by adding 1 part of water to 2 parts of a saturated solution; but it does not seem to give such good results as the chromic acid mixture.

9. *Osmic Acid.* This can be procured as a 1 per cent. solution in water, and it is then diluted to various strengths as required. It blackens fat and the medullary sheath of nerves.

A piece of mesentery placed in a weak solution for half an hour will show the fat cells lying along the course of the blood-vessels, as round black bodies.

It is also used for hardening the internal ear.

CHAPTER III.

On Cutting Sections.

Sections may be cut either by hand with a razor, or with a microtome.

1. In cutting sections by hand it is necessary to imbed the tissue in some material which will cut easily, and at the same time hold it firmly.

The best substance for this purpose is a mixture of wax and olive oil.

Take equal parts of white wax and olive oil by weight and melt them together, pour into a shallow vessel and when cold cut into small blocks.

Small tin-boxes with a removable bottom are required to hold the mixture while the tissue is being imbedded, and the best size is 2 inches long, by $\frac{3}{4}$ of an inch wide, and $\frac{3}{4}$ of an inch deep.

It will be necessary also to have a small porcelain ladle and a stand to raise it above a spirit lamp or gas jet. Melt some of the wax mass in the ladle and be careful not to make it too hot.

Prepare the tissue so that the face which is to be cut can be easily recognised, stick a needle into it away from the part which is to be cut, drain off most of the spirit by laying it on filtering paper, and then immerse in the melted wax mass, so that it is perfectly covered, take it out and let it cool. Take a small piece of filter paper and place it over the removable bottom

of the tin box, and then fix it in its place, the filter paper will prevent the wax from running out if the bottom fits loosely. It is also useful to leave a little of the paper projecting on which to write the name of the material imbedded. Then half fill the box with melted wax mass and hold the material in it, keeping it quite steady until the wax hardens, then by gently screwing the needle round it can easily be removed, and the box filled up with wax mass. It will be found a saving of time to imbed a portion of material at each end of the box. When the wax mass has become thoroughly hard, which will take some time, especially in warm weather, pull off the bottom and push the wax mass, with the filter paper adhering out of the box. It can then be laid by until wanted, the name of the material imbedded being written on the paper.

For cutting sections thus imbedded, a hollow ground razor is necessary; a very good one for this purpose can be procured from Baker, High Holborn; the razor must be very sharp. A small glass capsule about $\frac{3}{4}$ of an inch deep filled with methylated spirit is also required to put the sections into when cut, and to moisten the razor in.

Take the wax mass and with a scalpel carefully remove small slices from one end, until the imbedded mass can just be seen, then take the razor and dip it into the capsule, taking up a little spirit, let this run along the edge so as thoroughly to moisten it, and commence cutting as thin sections as possible, by drawing the razor diagonally across the mass with a steady sweep; this must not be done too quickly, and the

amount of pressure to be put on the razor will depend on the tissue imbedded and can only be learnt by practice. As each section is cut dip the razor into the capsule of spirit and wash it off. Wipe the razor occasionally and remove adhering portions of wax mass, and always keep the edge wetted with spirit. When a sufficient number of sections have been cut, the thinest should be selected and removed to a watch glass containing clear spirit.

Great care is required in cutting sections by hand, to hold the razor firmly yet lightly, so as to cut them thin and at the same time even, and this cannot be done without a great deal of practice.

For larger sections the boxes must be proportionately increased in size, and it will be found convenient when the wax mass is as wide or wider than the razor to cut off slices from each side so as to reduce the surface to be cut as much as possible without interfering with the stability of the imbedded material.

A small flat spear-headed needle will be found useful for taking up very small sections.

The Freezing Microtome.

A much easier method of cutting sections is by using a microtome. Of these there are a number made, in some of which the material is imbedded in wax mass, or a mixture of paraffin and lard, and raised gradually by a screw, while a razor is worked on a flat plate shaving off sections; these are also made with a chamber to contain a freezing mixture, so that the material can be imbedded and frozen.

Only one microtome will, however, be described in this work, as it is very simple and does the work well. This is Williams' microtome, made by Swift of University Street. It consists of a tub to contain the freezing mixture, with a brass standard into which screw the brass circular plates on which the material is frozen. A top with a glass surface fits on to this, having a hole through which the circular plate projects. The knife is fixed into a triangular frame, having screws at each angle by which it is raised and lowered.

To prepare the microtome for use :

1. Have the knife as sharp as possible.
2. Pound some ice finely in a cloth.
3. Scrape some salt into a fine powder.

With a spoon, put a layer of ice into the tub and then some salt on it, mix with the spoon, and so on, until the tub is about half full, then ram it hard with a stick and fill again, put in the salt and ice in about equal proportions, leave room for the top, wipe off the salt and ice from the edge, put on the top and fix it with the screw for that purpose. Screw the circular plate into its place, and the microtome is ready for use.

An India-rubber tube must be fixed to carry away the drainings as the ice melts.

To Prepare the Material for Freezing.

Any tissue which has been preserved in spirit, must be soaked in water for 24 hours to remove the spirit, and then placed in mucilage for another 24 hours.

It will be found a great saving of time, when a number of specimens are to be cut at one freezing, not to have the material too thick, as a piece a quarter of an inch thick will give an enormous number of thin sections, and take only a short time to freeze.

To make Mucilage.

Pour warm water on picked gum Acacia and make a solution rather thicker than the mucilages sold in the shops.

Cutting the Sections.

Remove all the specimens to be cut, from the gum, and place them in a small saucer ready at hand. Take up one with a pair of forceps and lay it on the circular plate of the microtome, drop some gum solution on it with a small brush, and see that it runs down on to the plate all round the specimen so as to fix it firmly. When it is thoroughly frozen adjust the razor so that it will just pass over without touching, and then lower the screw at the apex of the triangle by giving it a slight turn to the left and push the knife across the material in a diagonal direction. Have a small vessel ready containing warm *distilled water*. It is necessary to use distilled water, as in ordinary water the lime in solution is precipitated by boiling and the specimen will be covered by fine particles of carbonate of lime and utterly ruined. Moisten the upper surface of the razor with gum solution, and the sections as they are cut will slip up on it without curling, carefully remove them with a camel-hair brush and place them in the warm

distilled water and let them remain for 10 minutes or longer, until the gum is dissolved out; this will take longer with some material, such as testicle, than others.

With this microtome the most beautiful sections can be cut perfectly even throughout, surpassing anything that can be done with the hand. With care very large sections may be cut quite as readily as smaller ones, but to cut them well the razor must be very sharp, and the material not too hard; those hardened in chromic acid mixture, No. 1 (page 11) seem to do the best. Very little force is required in pushing the knife across the material, and if it is sharp a very slight turn of the screw each time will cut a section; these ought to be so thin as to be almost invisible, as the gum melts on the razor.

In cutting some material, such as retina, it is advisable to stain it *en masse* before freezing, otherwise the sections cannot be seen when placed in water.

CHAPTER IV.

On Staining.

List of Staining Agents.

1. Hæmatoxylin or Logwood
2. Carmine
3. Indigo-carmine
4. Purpurine
5. Anthra-purpurine
6. Eosin
7. Picro-carmine.

Aniline Dyes Soluble in Water.

8. *Soluble Aniline or China Blue
9. *Pure Soluble Blue
10. Serge Blue
11. Tyrian Blue
12. *Safranine
13. Dahlia, Rosanilin and Methylanilin Violet
14. Malachite Green
15. *Iodine Green
16. *Bismarck Brown.

Aniline Dyes Soluble in Spirit.

17. Spiller's Purple
18. *Rosein
19. Anilin Red
20. *Anilin Violet
21. Citranine
22. Aurine
23. *Pure Opal Blue.

Selective Stains.

Osmic Acid Chloride of Gold Nitrate of Silver.

To demonstrate the minute structure of any tissue, the sections require to be stained with some colouring agent which will show the different elements more plainly by their absorption of the colouring matter and bring out very transparent parts which otherwise would be hardly discernible.

Of all the staining agents, logwood is the most useful, and a solution prepared in the following manner will be found the most efficient.

Logwood Stain.

1. Take of

Extr. Hæmatoxyl, grms. 6
Alumen, grms. 18.

Mix thoroughly, while mixing add 28 c.c. of distilled water. Filter and add to the filtrate ʒ j of spirit of wine. Keep in a stoppered bottle a week before using. What remains on the filter can be mixed with 14 c.c. of distilled water, and left soaking in it for an hour or so, then filter and add to the filtrate ʒ ss of spirit of wine. This second solution is as strong as the first.

To Stain with Logwood.

Make a cone with a small round filter paper, and pour some of the staining fluid into it, let from seven to ten drops fall into a watch glass and dilute with *distilled water*. Let the sections remain in the solution for about a quarter of an hour, the time will depend on the tissue and the manner in which it has been hardened. Some tissues take in the stain very rapidly, others slowly. Take out a section from time to time, and place it in a watch glass of ordinary filtered water to see if it is stained deeply enough.

When the sections appear to have stained thoroughly remove them to a watch glass of ordinary water, and wash them to remove the excess of colouring matter. In staining with logwood it is necessary to be careful that too many sections are not placed in the solution at once, as they will lie thickly one on the other, and the staining will not be uniform ; it is also necessary to

dilute the logwood stain with distilled water, as ordinary water will not give the same result, owing to the different matters held in solution; but it is better to use ordinary water for washing the sections after staining as it helps to fix the color.

The solution should not be too strong, as better results are obtained from staining the sections slowly than from doing it rapidly, and it will always result in a loss of time if an attempt is made to stain a large number of sections in a strong solution.

On Staining Sections with Logwood that have been Prepared with Chromic Acid.

It is necessary to remove the chromic acid from sections hardened in that fluid, and this is done by taking some of the 5 per cent. solution of bicarbonate of soda diluting it slightly, and soaking the sections in it for some time, ten to twenty minutes will generally be sufficient.

Then remove them to plain water and wash well.

Prepare two watch glasses of dilute logwood stain and place the sections in one of them, let them remain for a minute and then place them in the other; there they must stay until stained deeply enough.

If the first watch glass be now examined, the logwood stain will be found to have become quite granular, and if this precaution had not been taken it would have been deposited as minute granules all over the sections.

2. *Carmine.* This stain, formerly so much used, is now almost given up by Histologists, as a general stain, in favor of logwood which is found to differentiate the

tissues much better while it has not the deleterious effect of carmine on the eyes when used with lamp light.

Carmine is, however, valuable in double staining, either as a carmine solution or in conjunction with picric acid as picro-carmine.

The carmine solution is prepared by mixing:

Carmine, ʒ ss.
Borax, ʒ ij.
Aqua, ℥ iv.

and pouring off the clear supernatant fluid. It must not be filtered.

3. *Indigo-Carmine* or *Sulphindigotate of Soda.* This is a useful stain in conjunction with carmine, especially for pathological specimens.

First make a saturated solution of the powder in distilled water and filter, take some of this solution and pour it into methylated spirit until it has attained a moderately deep color; a good deal of the colouring matter will be precipitated, and it must be carefully filtered to remove this, it is then ready for use. The solution should not have a very deep blue color and when held to the light, should show a purplish tint. It does not require long to stain the sections in it, and they should be allowed to remain long enough to stain them evenly, as it will be found that they stain first at the edges, after having been passed through dilute hydrochloric acid.

4. *Purpurine.* True purpurine is a red colouring matter extracted from madder, it is rather expensive

and not easily obtained. It is soluble in spirit with the addition of slight heat.

Place some of the powder in a test tube and add some rectified spirit, warm gently over a spirit lamp. When dissolved, filter and keep in a stoppered bottle. It stains readily and brings out some tissues such as muscle and mucous glands well.

It does very well in double staining with aniline blues.

5. *Anthra-Purpurine.* A brown powder which is often sold for purpurine. It is isomeric with it, but is found as a secondary product in the preparation of alizarin from anthracene.

It is very slightly soluble in water and may be removed entirely from aqueous solutions by means of ether.

A solution may be made by first dissolving it in spirit and then adding a warm solution of alum in water, the color will then become reddish. It stains muscle and epithelium but not connective tissue.

It does not show up minute structures as well as logwood.

It does not combine with picro-carmine in double staining, but the difference in the two colors is so slight as to make this of little value.

6. *Eosin.* Is not an anilin color but is a potash salt of resorcin. Resorcin is obtained by the action of melting potash on galbanum. Eosin is largely soluble in water and has a beautiful garnet red color. In using it a strong solution is required and the sections must be well washed in water after staining. It is a very

delicate color when used alone and almost too transparent, but in combination it becomes opaque.

This is the ordinary eosin, there is also an alcoholic eosin called primrose which is much used by silk dyers, it is soluble in a weak solution of spirit of wine, it fixes readily and gives a more brilliant color than ordinary eosin, and is not affected by light. There is also a blue shade of eosin which is said to have a great affinity for wool, and can be fixed readily by a solution of soda hypophosphis; this might be useful for investigations in diseases of the hair.

7. *Picro-Carmine.* This useful stain is difficult to make and the process is very tedious, it is better therefore to buy it prepared for use. It can be procured from Martindale, New Cavendish Street, at a reasonable price.

Filter about 10 drops into a watch-glass and dilute with distilled water. The sections must remain for some time, from twenty minutes to half an hour, and if at the end of that time they have not stained sufficiently, a little more picro-carmine may be added.

They are then placed in water acidulated with a few drops of acetic or picric acid and left for an hour.

When making experiments in treble staining a number of sections may be stained in picro-carmine and then placed in methylated spirit; there they may remain until required, as the spirit does not affect the stain, which forms a very good ground color, on which to try combinations of different anilines.

It is also a good stain for fresh tissues, such as mesentery when used with logwood. Also in sections of

skin it is very useful as a ground color, and by staining a number of sections of the same tissue with picro-carmine and then with two other colors, a variety of results will be obtained.

In some the tissue for which picro-carmine has a special affinity, such as connective tissue will be found unaltered, while the surrounding tissue has taken on the new colors. In others the picro-carmine has combined with one or both, and a new color is formed. Or again, it may be entirely supplanted by one of the other colors.

Anilin Dyes.

These may be divided into two classes, those soluble in water and those soluble in spirit; and this classification will be found very useful for experiments in double staining.

There are an immense number of anilin dyes now manufactured, but a great many of these will be found precisely similar in their colour and action, although bearing different names, while others are at the most only different shades of the same colour. The foregoing list gives a selection of the most useful of these dyes, and those marked with an asterisk will be found to give the best results. Many of the others would, however, be valuable stains if any means could be discovered for fixing them so that they could be passed through spirit without becoming obliterated.

8. *Soluble Anilin Blue or China Blue.* This is a useful stain for some tissues, such as stomach and spinal cord. It is made very simply by taking some of the

granules, and placing them in a large test-tube, and adding distilled water: the solution ought to be strong, nearly saturated. When all the granules have dissolved, filter the solution, and keep it in a stoppered bottle.

Sections that are to be passed through spirit require to be rather deeply stained, as a good deal of the colouring matter will come out in the spirit, and it is better to remove them at once from the water to absolute alcohol, as the colour is not affected so much by it.

9. *Pure soluble Anilin Blue.* This is a very good blue colour, soluble in water: it is more brilliant than the former, and more expensive; the China blue is, in fact, prepared from it. A saturated solution must be made and great care used in staining with it, as its action is very rapid. Like all anilin colours, its brilliancy is impaired by a long immersion in spirit.

This colour has not been much used as yet, but it will probably become a favourite when it is better known. To use either of these solutions, a few drops are placed in a watch-glass, and diluted with water, one or two drops are quite sufficient, the sections are then washed well in water.

10. *Serge Blue.* This is another shade of blue; a good colour, and stains well, but will not stand immersion in spirit.

11. *Tyrian Blue.* A much less brilliant colour than any of the others, more inclined to purple: it does not stand spirit, and cannot be much used until some method is found for fixing it.

12. *Safranine.* Is a good deep colour, and stains

gland tissue, such as mucous glands in the tongue, it does not, however, stand immersion in spirit well, and the sections require to be deeply stained. It has been recommended for staining amyloid degeneration, but does not excel several other anilines which can be more easily fixed.

13. *Dahlia. Rosanilin, Methylanilin Violet.* These three, differing but little in colour, may be considered together; they can be partially fixed by passing the sections through a 1 per cent. solution of hydrochloric acid, but even then they will not stand prolonged immersion in spirit, and it is better to remove them from the water at once to absolute alcohol. The solutions must be rather strong, and the sections well stained before they are removed to the acid solution.

14. *Malachite Green.* A very beautiful colour, but will not stand well: it is almost entirely removed by spirit, and is therefore not of much use to the Histologist.

15. *Iodine Green.* A darker green than the former, and very durable, standing spirit well and not fading, as far as two years experience goes. It is an invaluable colouring agent in double staining, as it is not so opaque as anilin blue. Make a saturated solution in water and filter, place a few drops in a watch-glass and dilute: it is very strong, and the section, when taken from spirit, will float on the surface, when it may be seen taking in the colour: if a light stain only is required it will be sufficient to let it remain on the surface; but if a darker stain is wanted it must be

wholly immersed, and then it must not be left long or the stain will be too deep, and it cannot be afterwards removed.

This is one of the most useful of the anilins soluble in water, and the results when it is carefully used are very beautiful; it picks out all the nuclei, and in growing bone it colours the unossified cartilage, giving a very striking result. It is also a most valuable anilin in double staining; its action will be described under that head.

16. *Bismarck Brown.* This is a good colour, a deep rich brown. It is easily soluble in spirit but does not make a very deeply coloured solution, it stains well. An aqueous solution can be made by the addition of a little dilute acetic acid, this solution also stains well but it remains to be seen whether it will fade or not. The aqueous solution stains gland tissue well, and in a section of tongue where the mucous glands are deeply stained with the aqueous solution, the colour has not faded after an exposure of two months. It is doubtful as yet which will prove to be the best solution, the aqueous is decidedly the richest colour to the eye but must be kept for a week, after making, before it is used.

Anilin Dyes Soluble in Spirit.

17. *Spiller's Purple.* This is a very good colour for use in double staining, but it is very difficult to fix it and if the section is passed through methylated spirit, it will be almost all washed out; it is not quite so easily acted on by strong absolute alcohol, ·795 sp. gr., but

the section must be very deeply stained to get enough left in it to make a satisfactory specimen.

There are several other colours soluble in spirit which are much easier to use and consequently this has not been much tried, but it would well repay the trouble if some means were found to make it more permanent. A saturated solution must be used, and it is better not to wash the section too much in spirit, before putting it in the aqueous solution in double staining.

18. *Rosein.* This is the best of all the anilin colours soluble in spirit and must not be confounded with rosanilin. It is easily soluble in spirit, the ordinary methylated spirit will do, its action is rapid, and it is better to use a strong solution and immerse the section for a very short time.

To make the solution, place some of the granules in a large test tube and nearly fill with spirit, cork and shake it and let it stand, when all the granules are dissolved, add some more until the solution appears to be saturated, then filter and keep in a stoppered bottle. Place some in a watch glass and dilute with about an equal quantity of spirit; this will do for all ordinary purposes.

19. *Anilin Red.* This is another shade of red and does not differ much from rosein in colour while it has not its universal applicability, and does not fix so well. It has not been much used, and experiments might bring out some valuable points in it as a dye for special subjects. It is prepared and used in the same manner as rosein.

20. *Anilin Violet.* This colour comes next to rosein

in utility while it surpasses it in beauty; it is not quite so easily fixed, otherwise it can be used in the same manner and as readily as rosein. It has this advantage over rosein, it is a much softer colour to the eye and makes a really beautiful combination with some of the aqueous solutions in double staining.

It is readily soluble in spirit, and even diluted makes a very opaque solution, rendering it a matter of difficulty to find the sections in it; they may be seen, however, by holding the watch glass against a lamp or bright sky.

Good results are often obtained with this colour by removing sections from water and letting them spread out a little on the needle in a strong solution and then removing them quickly to an aqueous solution of another color.

21. *Citranine.* A yellow substance insoluble in water and only soluble in spirit by the application of heat. It does not seem to give a deep enough colour to be a useful staining agent in microscopical investigations, but a more extended trial may bring out some valuable peculiarities, as it seems to differ a good deal from the other anilins.

22. *Aurine.* This is another colour of which there is not much known of its action on animal tissues; as far as it has been used, it has not given any satisfactory result.

23. *Pure Opal Blue.* This is the best blue soluble in spirit, and is therefore useful in cases where an aqueous stain is not admissible. It is made by dissolving the powder in spirit and then filtering it. Methylated

spirit will do if strong, but spirit of wine is better. A few drops are taken and diluted with spirit, and the sections immersed from three to five minutes, and then washed in spirit.

Selective Stains.

These will be found under their respective headings.

CHAPTER V.

Double Staining.

By double staining is meant a process in which two colours are taken, which have affinities for different elements in the tissues to which they are applied. Thus while one colour will stain the connective tissue and protoplasm of cells, the other will colour all nuclei and so differentiate the different elements as to make them more easily discernible. Others again will stain different glands according to their secretions. Thus showing a distinct chemical reaction between glands differing in their functions.

In other cases the duct of a gland can be stained of a different colour to the surrounding tissue and its own secreting substance, by which means it is easy to distinguish it, and thus show if it is implicated in any morbid change, and also in some cases prove whether the morbid change is primary in it or has extended from surrounding tissues, in which case all the ducts would not probably be similarly affected.

Double staining is a subject that requires to be very much more worked out than it has been hitherto, and in the present work, those processes only will be given in detail, which have been fairly well tried; many other combinations of staining agents will be apparent to the student, which have not yet been worked out, for want of time, and there is no pursuit in which patience

and time for experimenting are more required, than in double staining.

The student must not be discouraged by many failures as there is always some new fact to be learnt and noted, and in the application of these to future experiments, some brilliant results are sure to be obtained.

Picro-carmine and Logwood.

This combination has been used for a long time, and gives very satisfactory results. The student should begin with sections of scalp, skin, or tongue, and the result, if the process be carefully carried out, will be found very satisfactory.

The sections must be first stained in picro-carmine and then in logwood. Make a dilute solution of picro-carmine in distilled water, about 10 drops to the watch glass, and let the sections remain in it for from twenty minutes to half an hour, then wash in water and place in distilled water acidulated with 1 or 2 drops of acetic or picric acid. Let them remain in this for about an hour. Remove the sections from the acidulated water and place them in dilute logwood stain; this should not be too strong, from 5 to 7 drops to the watch glass of distilled water; do not let them stain too deeply. When sufficiently coloured, which will be shown by their becoming a faint lilac colour, they must be washed to remove the excess of logwood, and mounted in the usual manner.

This double stain is very effective when used with fresh tissues, such as serous membranes; it brings out

the connective tissue corpuscle in the mesentery of the newt, and at the same time shows the non-striped muscle tissue very well. It is also useful in bringing out the delicate tissue in the tubuli seminiferi of the testis and showing the developing spermatozoa there.

In any tissue when there are elements of different kinds, such as scalp or developing bone, it will be found to give very good results. The logwood stain must not be too deep, as it is a very opaque colour.

Carmine and Indigo-carmine.

This is a very useful double stain, and is especially applicable to sections made from material hardened in chromic acid mixture as they do not require to be passed through a solution of bicarbonate of soda, but can be placed at once in the carmine solution.

In this staining process three solutions are necessary.

I. Carmine solution (page 24).

II. Hydrochloric acid, 1 part; absolute alcohol ·795, 9 parts.

III. Indigo carmine solution (page 25).

Staining Process.

Take a few drops of No. 1 in a watch glass and immerse the sections, let them remain for two or three minutes and then remove them to a watch glass containing a small quantity of No. 2. Let them remain in this until they take on a bright rose colour, which will be in a few seconds, then wash them in methylated spirit to get rid of the acid. They must be washed in several changes of spirit. When the acid has been

thoroughly removed, place the sections in a little of No. 3 solution undiluted, and let them remain in it until they show a distinct blue tinge: the proper depth of this staining will be learnt by practice.

When carefully used this staining process is an admirable one, but there are one or two points that have to be attended to, or the two colours will not be sufficiently differentiated. If the section is left too long in the acid mixture the carmine will be taken out of the edges and these parts will afterwards take on the blue stain deeply, and so give a result the very opposite of that intended, as the whole value of double staining depends on one colour picking out the whole of one particular tissue throughout the section, and if this is not done the specimen is of no use. If the carmine stain is only just sufficiently acted on by the acid, so as to change the original dull purple colour to a bright rose, and the edges of the specimen are not bleached, it will, when put in the indigo-carmine solution, stain evenly throughout.

If the acid solution be too strong it will have the same effect as too long immersion in a weaker solution, and a few seconds will bleach the edges. This process will be found very useful in pathological investigation, as the carmine picks out very distinctly all the new growths.

Picro-carmine and Anilin Colours.

Some very good results may be obtained by staining sections first in picro-carmine, then letting them remain

in acidulated water for an hour, and afterwards staining them with various solutions of anilin colours.

Safranine, after picro-carmine, gives a good double stain, as the picro-carmine colours all the connective tissue and nuclei, while the safranine stains muscle, epithelium, &c.; but the two colours are not sufficiently different to give as good a result as logwood and picro-carmine, although they will be found useful where great transparency is desired.

Picro-carmine and iodine green give a very beautiful effect when it is wanted to isolate gland tissue; such as Peyer's patches, or the glands in the tongue, œsophagus, or solitary glands in the large intestine. The picro-carmine staining everything but the glands, which remain a bright green.

Eosin and anilin blue give good results, but require to be used cautiously, as if the staining is too deep the section becomes opaque. To get the best effect, the section should be very thin, and must be well washed after staining with eosin, and then just immersed for a few seconds in the anilin blue.

A great many other combinations will suggest themselves to the student, and he will be amply rewarded by experimenting further with the various staining agents mentioned.

Treble Staining.

The combination which has given the best effect so far in treble staining is: picro-carmine, rosein, and iodine green. Stain the sections well according to the process already described for picro-carmine, and soak them in acidulated water. Then take a few drops of

rosein solution, No. 18 dilute it with spirit, and immerse the sections for two or three minutes, remove them to methylated spirit and wash off the excess of colouring matter. Then place them in a dilute solution of iodine green. Coming from spirit they will float on the top of the watery solution, and this in many cases, when the green stain is not required to be very deep, is quite sufficient. When a deeper stain is required, immerse them altogether, and let them remain a minute or two ; but it must be borne in mind that this colour cannot be washed out again if too deep, which the spirituous stain can, so that it is better to have a section apparently over-stained in the rosein solution, while it is even under-stained in the iodine green. After washing, the sections are mounted in the usual manner. It will be found, however, that a good deal of the rosein will come out in the second immersion in spirit, and it is necessary to change it until no more colour comes away ; otherwise the oil of cloves will become coloured, and from it the Canada balsam, in which the specimen is mounted.

With the above mentioned three colours, the most beautiful effect may be obtained, but it will take some time and practice to get the process exactly right, and this is a matter which can only be gained by experience. The results will be found to vary with the length of time the section is immersed in each of the two last colours, and also with the strength of the solutions.

The section should be uniformly and deeply stained with picro-carmine. The other two solutions should be

saturated in the first instance, and then diluted one-half at least. If they are to be laid aside for some time before mounting they should not be left in spirit, but in oil of cloves. Only a few sections should be stained at one time or some will be found much more deeply stained than others. The best results will also be obtained with material that has been hardened in chromic acid.

This staining process is well shown in a section of the base of a cat or dog's tongue, cut through one of the circumvallate papillæ, the section should be sufficiently large to include some of the mucous glands, of which there are a large number in that region.

If the staining is well done it will show all the muscle fibres stained with picro-carmine, the connective tissue, protoplasm of cells, &c., stained with rosein; while all the nuclei in the superficial epithelium, serous glands, non-striped muscle tissue in the vessels, and elsewhere, are stained a brilliant green.

The most important fact demonstrated by this process is the different chemical reaction shown by the various glands. In the mucous glands, while the epithelium lining the duct is stained in precisely the same manner as the superficial epithelium of the organ, it will be found that the moment the secreting epithelium is reached a new colour presents itself, which differs *in toto* from either of those employed in the process; thus showing that the secretion has the power of causing these two colours, green and red, to combine, forming different shades from purple to blue,* according to

* Iodine green is a very blue green.

which colour predominates. In the serous glands, however, quite another aspect is presented; there is no combination as in the mucous glands, but the protoplasm of the cells is stained more or less deeply with rosein, while the nuclei have taken on the green; the colour differs, however, from that of the surface epithelium, and appears to have taken on picro-carmine to some extent, which, with the rosein, gives a dull red colour.

In many places will also be seen small masses of adenoid tissue which have stained a bright green throughout.

Altogether this makes one of the most brilliant specimens in the whole range of histology, and although the process is rather troublesome, and requires a certain amount of practice to determine the time required for each immersion, it amply repays when once properly done.

Take only a few sections at a time, and do not hurry over the different processes, and after a few trials the exact time of immersion will be hit on, and should be recorded.

Chloride of Gold and Anilines.

Some very striking results may be obtained by first staining fresh tissues, especially growing bone, in chloride of gold solution (page 7), and then decalcifying and hardening in spirit. After the material has hardened sufficiently, sections may be made and stained with two colours. It is not quite clear what action the gold chloride has on those parts it does not stain, but

that it has some, is evident from the difference of the action of anilin dyes on those specimens prepared in gold, from those hardened in any other manner.

A very good material for the purpose is the tail of a young rat or mouse, placed in half per cent. solution of gold chloride for an hour or two, and then decalcified and hardened in the usual way. Very thin transverse sections should be cut, and stained first in rosein and then in iodine green.

On examining the specimen the gold staining will be seen on the periphery, bringing out the tendon cells, and giving a dark hue to everything for a certain distance from the outside; but within this a great variety of colour will be found, the different tissues being stained in a most gorgeous manner. In the middle the bone trabeculæ will be seen faintly stained, while the calcified cartilage, in their centres, is stained a bright colour, totally different. All these colours may be varied by using different anilin solutions, and a very pretty result may be obtained by simply staining with iodine green. In the above instance the true bone is only faintly stained, while the calcified cartilage takes the colour deeply; this may be reversed by using the carmine and indigo-carmine process after the gold, when the bone will be found deeply stained, while the calcified cartilage is not stained at all, and looks like a clear space in the bone trabeculæ. These two processes will be found invaluable in any investigation into the development of bone.

CHAPTER VI.

On Mounting.

Slides.

Glass slides, 3 × 1, must be cleaned before using, and a good plan is to keep some cleaned, ready for use, in a two-dozen box with rack work, where they stand on their edges and do not get dusty. The ordinary slides sold at the shops at 6*s*. a gross, are easily cleaned with a chamois leather. Sometimes, however, especially when using slides for the second time, they cannot be cleaned so readily, and they must be soaked in a decoction of oak galls for some hours; this is made by pouring boiling water on bruised oak galls and straining.

Cover Glasses.

The usual size of these is $\frac{3}{4}$ of an inch square, but larger ones will be required, and some of $\frac{7}{8}$ of an inch should be obtained. For practical men square cover glasses are the best; but where æsthetic mounting is the order of the day, round cover glasses and variously coloured cements for sealing, with the help of a turn-table will be required; these, for obvious reasons, will not be considered in this work.

Thin glass is made by Messrs. Chance, of Birmingham, in three thicknesses, designated by the numbers 1, 2, and 3; of these No. 1 is the thinnest. This is the best to use. It varies in thickness from ·004, or even thinner to ·008.

On Measuring Cover Glasses.

For ordinary students' work the No. 1 cover glasses will do perfectly well, as they are very near the thickness to which object glasses, without a correction collar, are adjusted; but when high power glasses are to be used, it facilitates the work very much, to know the thickness of the cover glass under which the specimen is mounted, and with very high powers, or those with wide angle of aperture, the cover must be at least ·004 to enable the glass to work through it. Powell and Lealand's $\frac{1}{25}$ water immersion requires a cover glass ·003 of an inch.

To save expense, as dealers charge a high price for very thin measured glass, the student will find it a great advantage to purchase a small American steel gauge, sold by Buck, Holborn; it is made for measuring the thickness of sheet brass in fine work, but does admirably for measuring cover glasses; the way to use it will be at once apparent. With this instrument let the student measure an ounce of No. 1 glass, and select all those that are ·004 and under, they may be put away for future use. This is not much trouble, and a few of these very thin glasses will last a long time, as it is only in special work that they will be required. The oil immersion lenses have also done away in a great measure with the necessity of using measured cover glasses.

On Cleaning Cover Glasses.

The following plan will be found a very good one, both in the saving of time and breakage.

Place the cover glasses to be cleaned in a glass vessel containing strong sulphuric acid, and agitate gently until the acid has penetrated between the glasses and driven out the air-bubbles, let them remain in this for an hour or two and then wash well in water until no acid is left. Remove them to a capsule containing methylated spirit. Take out each one separately with a pair of broad pointed forceps, and wipe dry with a silk or soft linen rag.

With very thin cover glasses, such as ·003, each glass may be dipped in absolute alcohol when taken out of the methylated spirit and then carefully dried with an old silk handkerchief.

Mounting Fluids.

For fresh tissues :

Glycerine.

For hardened tissues :

Canada balsam solution.
Dammar varnish.

Mounting Fresh Tissues.

Place the tissue to be mounted in a capsule of water of sufficient depth to cover more than half of an ordinary glass slide, when placed in it with one end on the bottom and the other resting on the opposite side. With a needle, bring the tissue over the middle of the slide and hold it there, at the same time raise the upper end of the slide very gently, so that the tissue will adhere to it and be raised out of the water. See that it

is not folded in any part. Lay the slide on some filter paper, and with needles spread out the tissue to its fullest extent, without stretching it. It is necessary to be very careful of this, as if the tissue be a serous membrane, stained with silver, the outlines of the cells will be completely destroyed wherever it has been stretched. In the same way, non-striped muscle fibre in the mesentery of the newt, will be broken up and quite ruined.

When the tissue appears to be extended in a natural manner, without folds, take up the slide and wipe off all moisture from it with a clean cloth, if there is a large quantity on the specimen, some may be removed with a bit of filter paper, but great care must be taken not to touch the specimen itself with the paper as it will adhere to it; at the same time it must not be allowed to become dry, and if this seems probable, it can easily be moistened by breathing on it occasionally, until the cover glass is ready. Take up a clean cover glass and place a drop of glycerine on the centre, invert and place it horizontally on the specimen, leaving the weight of the cover glass to spread out the glycerine. If there is an excess of glycerine round the edges of the cover glass, it must be removed by placing small pieces of filter paper in contact, which will soon absorb the superfluous fluid, but must not be left too long or they will drain it from under the cover glass. When sufficient has been taken up by the pieces of filter paper, remove them and wipe the slide with a dry cloth, taking care to clean off all glycerine without touching the cover glass.

When this is done the preparation must be sealed, by

painting round the cover glass with either Dammar varnish or Hollis' glue, taking care that only the extreme edge of the cover glass is included. It will be necessary to give a second and third coat if Dammar varnish is used, at intervals of a few days.

It will be found a good plan to seal first with Dammar varnish, and afterwards to cover this with Hollis' glue, as it makes the preparation more secure, and it is absolutely necessary to have them sealed with Hollis' glue when oil immersion lenses are to be used, as the cedar oil does not touch it, while it dissolves Dammar varnish at once.

Specimens carefully prepared in the above manner may be kept for years without deteriorating.

Mounting in Canada Balsam or Dammar.

Canada balsam mounting fluid is prepared by mixing :

Canada balsam, 105 parts.
Turpentine, 35 parts.
Chloroform, 35 parts.

Dammar varnish is prepared thus :

Take of

Gum Dammar in powder, ½ oz., and dissolve it in turpentine, 1½ oz. Filter.

Gum mastic, ½ oz., and dissolve it in chloroform, 2 oz. Filter.

Mix the two solutions and filter again.

Put in stoppered bottles, and see that they are perfectly free from moisture before using. A small drop-bottle of each of these fluids must be kept for daily

use, and when they get thick from evaporation a little chloroform can be added.

Both of these mounting fluids are used in the same manner and one description will apply equally well to each. Canada balsam is the one commonly used, as the materials of which it is composed are very cheap, while gum dammar is rather expensive. The Dammar varnish is also sometimes apt to become cloudy after a time and it is difficult to make.

To Mount in Canada Balsam or Dammar Varnish.

The sections having been properly stained and washed, are placed in methylated spirit to remove some of the water, and then immediately transferred to a small quantity of absolute alcohol in a watch glass, and covered with another to prevent evaporation. They should be left in this for about 10 minutes. The absolute alcohol, which should be the strongest sp. gr. ·795, has a great affinity for water, and will remove all that is in the sections.

When ready remove the sections one by one from the absolute alcohol with a needle, and drain off as much alcohol as possible by touching the section on the back of the hand or on a piece of clean filter paper, the back of the hand is the best, as some fibres from the filter paper may adhere to the section, which when seen under the microscope, will not improve the beauty of the preparation; when sufficiently drained, without being allowed to become absolutely dry, they are placed in a vessel containing oil of cloves; they will spread out on the surface of the oil, and as the spirit evapo-

rates they will become completely permeated with it and very transparent. If there are any folds these should now be straightened out carefully with needles.

Having placed a drop of Canada balsam on the slide, spread it out slightly with a needle, select a section in the oil of cloves, and pass the copper lifter under it, raise the lifter and hold the section in position with a needle by its upper corner, and having made sure there are no folds, remove the lifter with the section on it from the oil of cloves, let as much oil drain off as possible against the side of the vessel, and remove the rest by placing the edge of the lifter on a piece of filter paper. Place the edge of the lifter on the slide in the drop of Canada balsam, and gently draw down the section with a needle as soon as a corner projects from the lifter on to the slide, hold it there lightly with the needle and slowly draw away the lifter; if this is carefully done the section will lie in its place in the middle of the slide without any folds.

A lifter is made by beating out the end of a copper wire, filing it smooth, and then turning up the broad portion slightly.

Take up a cover glass with the broad pointed forceps and hold it between the thumb and fore-finger of the left hand, place a small drop of Canada balsam on its lower edge, transfer to the right hand and gently lower it on to the section, keeping the left thumb against one corner to prevent its slipping, and gradually lower it by removing the right hand very slowly, watching all the time to see that no air bubble is entangled in the section.

With a little practice this can be done very neatly without an air bubble in any part of the preparation; it requires patience, however, and it is no use to try air pumps or any dodges, to remove the bubbles, as they are useless; the only thing to be done when an air bubble lodges in a cavity of the section and refuses to move in any way by gentle pressure, is to lift the cover glass, and transfer the section to oil of cloves, and then remount it.

When several sections are to be mounted on one slide, a slight pressure on each with the needle will generally retain it in its position, if too much of the mounting fluid is not used.

It will often be found on examining preparations after they have been mounted some little time, that the fluid has evaporated and left a vacuum under the cover glass; in this case a drop of the mounting fluid must be placed on the slide in contact with the cover glass, and it will immediately run in and fill up the empty space, provided always an egress has been allowed to remain for the contained air, when this is impossible from the small size of the hole at the edge of the cover glass, the only thing to be done is to wait until some of the material, of which the mounting fluid is composed, has been dissolved by the fresh fluid. Applying heat will effect it, and at the same time in all probability ruin the specimen.

Each preparation should be examined under the microscope and if found to be worth keeping, labelled. On the label should be noted the tissue, date of its preparation, mode of hardening and staining, thickness of

cover glass if it has been measured, and anything of note which may be seen at the time it is examined. Exceptionally good sections should always have a private mark to show that they are not to be given away or exchanged.

They should be kept in a cabinet where they may lie flat.

On Breaking down Old Preparations.

It is often necessary to break down an old preparation and remount it. The cover glass may be broken, the staining faded, or the cover glass may be too thick, and preparations should never be discarded for these reasons, as it is quite easy to remount them. When a specimen has been mounted in glycerine, it is an easy matter to remove the cover glass, all that is necessary being to cut round the cement with a sharp knife, lift the cover glass carefully with a needle, and float off the section in water; if it is very delicate the cover glass had better be removed under water. The section can then be washed, to remove the glycerine, and restained if required; it will then be ready for mounting in the usual manner.

To break down a specimen mounted in Canada balsam or Dammar varnish is more difficult, especially if it has been mounted long enough to allow the balsam or Dammar to become hard. It must be placed in a bath of chloroform until it becomes soft enough to remove the cover glass, and this may be facilitated by passing the slide over the flame of a spirit lamp so as to heat it very slightly, but this requires care as the section may be easily ruined.

After the cover glass has been removed the section must be floated off into chloroform until all the balsam or Dammar has been dissolved out of it, and then placed in alcohol for a short time; it may then be re-stained if necessary and mounted again.

Mounting Large Sections.

In manipulating large sections it is rather difficult to pass them through the different processes without injury. This may generally be done with care, and they may even be double stained.

There are some tissues, however, so fragile that they cannot be lifted on the needle without tearing, and these must be left in one vessel and the different processes applied to them there. This is not a very satisfactory method, as the staining cannot be so well done unless a large quantity of fluid is used, and every section carefully separated from its neighbour, they are also apt to be injured in pouring off the different fluids. This is only required in exceptional cases. It is when the mounting from the oil of cloves comes, that the difficulty is experienced; as, even if a lifter is specially made large enough to take up a whole section, the adhesion of the section to such a large surface is so great, that it is impossible to get it off without tearing, if the section is as thin as it ought to be. It may, however, be done by using the cover glass as a lifter in the following manner.

Take as an example a longitudinal section of kidney of large dog or man, having been safely stained, it lies in the oil of cloves ready to be transferred to the slide.

The section measures say about 1¾ inches by 1 inch, some slides must be procured 3 by 2 inches and some cover glasses 2 by 1½ inches, these had better be of No. 3 thin glass.

Having cleaned one of the slides, place some Canada balsam on it and spread it out with the needle, to something near the size of the specimen, then take the cover glass and pass it into the oil of cloves under the specimen, in the same way the copper lifter is used to smaller sections; lift the cover glass and keep the section in its place, then drain off the superfluous oil by holding the cover glass on filter paper, on lifting it fresh from the oil it should be allowed to drain slowly from one corner, then invert the cover glass with the section on it, place a little Canada balsam at the lower edge, and lower it gently into the Canada balsam on the slide; this must be done very carefully, as bubbles will be found here and there, and the cover must be lifted a little and lowered again, until they have all been driven out. It is a tedious process but amply repays the trouble.

The great drawback in this method is that the front of the cover glass is covered with oil of cloves and cannot be cleaned until the balsam sets, a matter of time with such a large surface. It can certainly be sealed up with Hollis' glue, but even then it is not safe and requires a great many coats before the glue is sufficiently strong to resist such pressure as is required to clean the cover glass.

With some tissues it is possible to use a large cover glass as a lifter, and by allowing a large quantity of oil of cloves to remain to draw it off on to the slide and cover

in the usual way, but with other tissues such as testis this is utterly impossible if the sections are thin, and they can only be mounted in the manner first mentioned.

Thin Slides.

For preparations to be examined, under very high powers, thin slides made from glass called 9 or 10 oz. crown are useful, as they allow the condenser to come close to the object.

CHAPTER VII.

Method of obtaining Animal Tissues for Examination.

The animals required will be a cat, rabbit, and guinea pig; and for some special tissues, a frog, salamander, and newt.

To kill the first three animals, place them in a box with a tight-fitting lid, having previously introduced a sponge saturated with chloroform. In this confined space the chloroform will render the animals insensible in a very short time, and then kill them without inflicting the slightest pain.

The other three animals may be chloroformed under a small bell glass on a plate.

Having killed either of the first animals, make an incision through the skin, from the chin to the anus, and reflect it on each side, open the abdomen and remove some of the mesentery and omentum for preparation by the silver process; open the thorax and make an incision into the heart and drain off the blood before it coagulates. Before opening the heart a small piece of the centrum tendineum of the diaphragm may be carefully cut out to be prepared by the silver process. Then open up the thorax and abdomen thoroughly. Take out the lungs with a small portion of the trachea attached. Remove the heart, open up the ventricles. Remove the liver and cut it into small pieces. Take out the stomach with a piece of œsophagus and duodenum attached, open it longitudinally and wash it in dilute

solution of chromic acid to remove particles of food, &c.; this must be done gently, and it must not be rubbed. Open the intestines with scissors, and wash in dilute chromic acid; cut into short lengths. Cut out carefully the ilio-cœcal valve with a portion of intestine on either side. Remove the kidneys, and open one longitudinally, the other transversely, in two or three places. Take out the spleen and pancreas and cut them in small pieces. Take out some of the mesenteric glands; remove carefully the uterus and ovaries if a female. Open the scrotum and remove the testes, in many cases these can be got out more easily by pushing them up into the abdomen. Dissect out the penis and remove it. Then cut round the skin of the neck and disarticulate the skull, taking care to leave the remaining portions of the trachea and œsophagus. Disarticulate the lower jaw and remove the tongue with the trachea and œsophagus; cut off these at the base of the tongue, then divide that organ longitudinally, leaving one side whole, make transverse cuts in the other. If large it will be necessary to make some longitudinal incisions in the first for hardening with chromic acid; but this should be done so as not to interfere with making longitudinal sections of the whole of one side. Take out the submaxillary glands. The eyes and brain will then only remain, and great care must be exercised in taking them out.

The best plan is to remove the brain first; for this purpose take off the covering of the nasal organ with a pair of bone forceps, and snip off pieces of bone from the upper part of the skull, taking care not to touch the

brain underneath, at the same time care must be used not to squeeze the eyes in holding the skull. When all the upper part of the brain is laid bare, the bone should be nipped through in front of it with the forceps, and the base of the skull divided; by gently cutting away the bone by degrees and dividing the nerves proceeding from the brain, it can be completely freed with the cerebellum, pons, and medulla intact.

It requires some practice and care to do this well, but even if the brain is a little torn in removing it, there will still be plenty to harden and make sections of. The bone can now be cut through, round, and in the orbit the optic nerve and recti muscles divided, and the eye removed whole.

Portions of nerve can be taken from different parts, the optic, sciatic, &c. Portions of muscle may also be cut out. The aorta and some of the larger arteries should be removed and cleaned from the surrounding tissues.

Dissection of Frog.

The whole eye may be removed from a frog and placed in Muller's fluid, but the cornea is required to make a gold preparation.

Having killed a frog, wrap it in a cloth and render the eye tense with the thumb of the left hand. Insert one of the points of a fine pair of curved scissors at the edge of the cornea and cut it round carefully, separate it from the rest of the eye, and place it in gold chloride.

The mesentery and meso-gastrium may be prepared by the silver process.

Dissection of Newt and Salamander.

Kill a newt and cut off the head, fix it to the table on its back, by a needle passed through the neck and another through the tail. Make a longitudinal incision down to the anus, take up the upper end of the stomach with a pair of forceps, and by carefully cutting away the connective tissue attaching it to the cavity, the whole of the contents of the body can be removed *en masse*.

Cut away the lungs and liver, which are not wanted. Make an incision through one side of the stomach in its whole length, and then put the whole in solution of chromate of ammonia. In Triton cristatus there will generally be found in the male, two testes on each side, but sometimes three or four; some of these may be removed for the purpose of making a fresh preparation of Spermatozoa.

Salamander is treated in the same way for the same tissues.

PRACTICAL HISTOLOGY.

BLOOD.

Take a small drop of newt's blood, cover and examine: notice the difference in shape and number between the coloured and white corpuscles. Prick the finger and examine human blood in the same way.

Amæboid Movement.

A warm stage is required to show the amæboid movement of the white blood corpuscles.

With a camel's hair pencil apply a little oil round the edge of a cover glass, place a small drop of perfectly fresh newt's blood in the centre of the glass, and cover with another; lay the preparation on the warm stage over the central hole, and apply the spirit lamp to the wire. The thermometer should rise to 30° C.

Select one of the large colourless corpuscles, and sketch the different movements shown by it at distinct intervals. Make a similar preparation of human blood, and examine in the same manner. The cover glass must be warmed for human blood, and the top cover glass should be touched on the spot of blood coming from the pricked finger. The coloured blood corpuscles will form rouleaux if properly prepared.

A preparation of fresh newt's blood should be examined with a high power on the warm stage to see the

beautiful intra-nuclear and intra-cellular network of fine fibres in the red corpuscles.

Feeding Blood Corpuscles.

Rub up vermilion cake in $\frac{3}{4}$ per cent. salt solution, add a very small drop of this to the blood on a slide, cover and examine on the warm stage, paint a little oil round the edges to prevent evaporation.

In the case of newt's blood the thermometer should not rise above 30° C., and in that of human, not above 40° C. After a time the white blood corpuscles will be found to have enclosed some of the vermilion granules. Newt's blood from the larger size of the corpuscles will show this best.

Irrigating blood corpuscles.

Make a $\frac{3}{4}$ per cent. saline solution of newt's blood on a slide, cover and examine: notice the red corpuscles gradually becoming crenated. Try the effect of irrigation on this preparation, place a small piece of filter paper in contact with the edge of the cover glass, and with a capillary tube place a small quantity of the fluid to be introduced on the opposite side; as the filter paper withdraws fluid from one side, the new fluid will flow in from the other. When thicker fluids, such as glycerine are to be removed, a capillary tube is necessary on one side to remove it by suction with the mouth.

The fluids to be tried by irrigation are:—Dilute acetic acid; distilled water; 2 per cent. solution Tannic acid; 2 per cent. solution Boracic acid. For their effect the student is referred to the *Atlas of Histology* by Dr. Klein.

Hæmin Crystals.

These are interesting as demonstrating the presence of blood, and they are very easily prepared, and can be sealed up and kept as a permanent preparation. Glacial acetic acid decomposes the blood pigment, and forms a hydrochlorate of hæmatin in the presence of sodium chloride.

Place some ordinary table salt in a watch glass, and hold it over a spirit lamp until it is thoroughly dried. Take equal parts of any dried blood and this dried salt on a slide, put on a cover glass, and with a capillary tube run in glacial acetic acid, warm it gently over a spirit-lamp until a good deal of the acid has evaporated, and then examine under the microscope. To mount this as a permanent preparation, wash out with distilled water all the glacial acetic acid, and seal with Hollis' glue. It is better to use some rather largish masses of blood, or when the acid is being washed out all the crystals will go as well, the larger masses will be held by the weight of the cover glass, and their margins will be found covered with Hæmin crystals.

EPITHELIUM.

Squamous Epithelium.

With a blunt knife scrape a little saliva from the back of the tongue or inside of the cheek, cover and examine. Look for squamous epithelium, the so-called salivary corpuscles showing Brownian movements of the granules they contain, micrococci and in many cases bacteria.

Shed skin of newt. This makes the best preparation of surface epithelium. Place a newt in a glass jar of water, and in three or four days it will be found that it has shed the entire outer layer of epithelium as a continuous skin; unroll it carefully, and cut into small pieces, stain with logwood and mount in glycerine. The staining will be facilitated by first placing it for a short time in slightly acidulated water. Seal with Hollis' glue, and examine with a low power. The outlines of the cells and their nuclei will be very well shown.

Make a vertical section of skin prepared in chromic acid mixture (wherever this mixture is mentioned, solution No. 1, p. 11 is meant). Stain in logwood and mount in Canada balsam.

Observe the different layers of cells, and with a high power seek for the prickle cells of the Rete Malpighi. These are, however, better seen in some epithelial Cancers.

Columnar Epithelium.

Take the stomach of a newt or salamander prepared in solution of chromate of ammonia (page 13), and wash well until no colour comes away, place the whole in a dilute solution of picro-carmine, about 15 drops to a watch glass of distilled water, and let it remain until it has taken on a deep red colour, then remove and wash off the excess of colouring matter; scrape off a little of the surface material from the inside of the stomach, and tease out gently on a slide in a drop of glycerin, cover and examine.

With a high power the network in the cells and their nuclei will be seen as depicted in the Atlas.

Make a longitudinal section of the large intestine of cat, dog or rabbit, prepared in chromic acid mixture, stain with logwood, mount in Canada balsam, and examine. The columnar cells will be seen in rows, some of them having become goblet shaped, that is distended by their secretion, and if the animal has been killed some time after feeding, when digestion is going on, these goblet cells will stain deeply with logwood, and the mucin will be seen poured out from the cell in deeply-stained masses. If, however, the animal has been killed within a short time of feeding, that is before the mucigen has been changed into mucin; these goblet cells will not stain with logwood but will stain with anilin colours.

Ciliated Columnar Epithelium.

Take a portion of the trachea of cat, prepared in 2 per cent. solution of bichromate of potash, wash well in water, and stain the mass in logwood; it must be put in a strong solution and left in for several hours; when deeply stained remove it, wash well until no more colouring matter comes away. Then with a small knife scrape away a little of the inner surface, and place it in a very small drop of glycerine on a slide, pound it with the rounded end of a needle holder until the whole drop is seen to be permeated with fine particles, and no large ones are left; place the cover glass on gently and allow the fluid to spread under it; seal and examine.

The ciliated cells will be found isolated and can be readily examined. Two other varieties of cells will be found, which are the cells of the deeper layers and they are not ciliated; try with the $\frac{1}{6}$ to make out the striation of the line running across the cell at the base of the cilia; a good glass will show this; the $\frac{1}{12}$ oil immersion will show that this striation is caused by the cilia, which are continuous with the longitudinal striation in the body of the cell.

Make a transverse section of the epididymis of man or dog prepared in chromic acid mixture and stained with logwood. Here the ciliated cells will be beautifully shown, the cilia being much longer than in the trachea; they can be traced through the striated border, with a high power in the same manner, as in the cells of the trachea.

Endothelium.

Take a portion of mesentery of cat and prepare by the silver process. When it has been left long enough in distilled water, stain it with logwood and mount in glycerine; seal and examine.

The nuclei will be well shown by the logwood stain, but the outlines of the cells will not probably be very distinct at first, but will become more so by the action of light. It will then be seen that the silver has been deposited in the intercellular substance between each cell, giving it a dark border. By focussing carefully a second layer of cells will be brought into view, whose outlines do not correspond with the first; these are the endothelial cells on the other surface of the membrane.

In some places masses of small cells will be found deeply stained, these are germinating cells.

Other serous membranes should be prepared by the silver process, the centrum tendineum of the diaphragm makes a very good preparation, showing the groups of germinating cells, and the difference in shape of the endothelium on each surface.

The lymphatic capillaries will also be seen by the different shape of the endothelium, giving them the appearance of trabeculæ running through the membrane.

A silvered preparation of the septum cisternæ lymphaticæ magnæ should be made.

Remove the viscera from a recently killed frog, and a large lymph sac will be found on each side of the spinal column behind the stomach, the septum separates each sac from the peritoneal cavity. It may be stained in situ by pouring a little ½ per cent. solution of nitrate of silver over it and allowing it to remain a few minutes, or the septum may be carefully removed and placed in silver solution. The septum will show the germinating cells round the opening of the stomata. In all cases where preparations of serous membrane are to be made, the animal should be bled first.

Connective Tissue Corpuscles.

Make a gold preparation of Tadpole's tale.

This will show the connective tissue corpuscles very well with their branched processes. Pigment cells are also numerous. Several of these preparations should be made, as they show a great many different structures.

Make a preparation of Newt's mesentery in 5 per cent. chromate of ammonia (page 13), and double stain with picro-carmine and logwood (page 36). In this preparation very large branched corpuscles will be seen having the hyalin ground plate stained with logwood; these make most beautiful objects for examination with high powers.

The corneal corpuscles will be mentioned in another place.

Tendon.

To show the tendon cells which lie in the interfascicular lymph spaces, take a young mouse just killed and remove the skin of the tail, then with the fore-finger nail separate two of the caudal vertebræ and forcibly remove the distal portion. Several white threads will be left, these are the tendons. Take a small bit of one of the finest and place it in slightly acidulated water for a short time, then remove to half per cent. gold solution, let it remain about twenty minutes. Then place it in distilled water, which must be changed once or twice, until it becomes a bronze colour. Take a small bit and place it in a drop of glycerine on a slide, and separate it into as many fibrils as possible. Cover and examine.

Take the tail of a young rat and prepare it in gold chloride (page 14). Make transverse sections and double or treble stain them. The tendon cells will be seen darkly stained, with the gold lying between the bundles of fibrous tissue forming the tendons.

Tendon should also be examined in the fresh state,

by taking a small portion from the mouse's tail and mounting in salt solution, then irrigating it with very dilute acetic acid, and watching the change that takes place, as the fibrous tissue swells up and becomes indistinct, the cells becoming granular.

The acetic acid should be only just sour to the taste.

After a time the whole of the fibrous tissue will have disappeared, leaving a very few elastic fibres which are untouched by the acid.

Take also some of the fresh tendon and place it in logwood stain, to which a few drops of glycerine have been added; let it remain until deeply stained. Tease out small portions in glycerine on a slide; the tendon cells will be well shown by this process.

Elastic Tissue.

Make a preparation of mesentery of frog and mount in glycerine; a very fine network of elastic fibre will be found throughout the whole structure.

Take a small slice of the ligamentum nuchæ of the ox, which can be readily procured from the butcher's. Place it in dilute acetic acid for some little time, until it swells up, then tease a small portion in a drop of glycerine on a slide. Cover and examine.

White Fibrous Tissue

Is well shown in many of the preparations of serous membranes. A special preparation should, however, be made, by hardening omentum in 1 to 2 per cent. bichromate of potash and staining with logwood; it will show the large amount of fibrous tissue present in

a serous membrane, forming the greater part of the framework.

White fibrous tissue is also well seen in sections of skin; also in submucous tissues.

Adipose Tissue.

Well seen in some of the preparations of serous membrane, when the fat cells lie thickly along the sides of the blood vessels. Also seen in cutis vera, and in many other parts. It is not necessary to make a special preparation of it.

A serous membrane placed for a short time in dilute osmic acid, and mounted in glycerine, will show the fat cells differentiated from the surrounding tissue, as they have all become blackened by the action of the osmic acid.

CARTILAGE.

Hyaline Cartilage.

The thin cartilaginous expansions from the sternum of the newt, prepared by the gold process, make very good specimens of hyaline cartilage. Thin sections may be cut by the microtome, or by hand. In this preparation the lymph canals will be seen looking like dark processes proceeding from the lacuna, in which the cell lies, into the hyaline matrix.

Sections of the nasal cartilages of small animals, growing bone, &c., will all give good examples of hyaline cartilage.

In the fresh state the cells will be seen to fill the

lacunæ; but in hardened specimens they have all shrunk, more or less, leaving a space.

Specimens of cartilage should also be hardened in chromic acid mixture, and thin sections stained with logwood.

Fibro-Cartilage.

Make a longitudinal section of mouse's tail, and notice the intervertebral cartilage and its gradual transition into hyaline cartilage on the bone.

The fibres will be seen in various aspects as they cross one another, and several sections should be examined; the cells will be seen lying between the fibres. Make a section of the intervertebral disc of sheep or ox, hardened in chromic acid mixture, and stain with logwood. Mount some sections whole and tease out others on the slide. Cover and examine. It will be difficult to make out the fibres in some sections.

Elastic Cartilage.

This can be well shown in the lobe of the ear or in the epiglottis. Procure the epiglottis of a sheep and harden it in the chromic acid mixture, cut sections and stain them with logwood.

The ear of a child prepared in the same manner, sections cut and stained with logwood.

The ear lobe of a pig also prepared in chromic acid mixture and stained with logwood. Sections of these must be thin and they must not be hardened too much or it will be difficult to cut them.

The pig's ear when well prepared makes a very useful specimen, as it shows a great many tissues.

BONE.

Preparing Hard Bone.

Bone must be examined in two forms, first in its dry state, and secondly when it has been decalcified or had its earthy salts removed. In the case of dry bone a very few sections will suffice as it is a difficult and laborious task to get them well made.

The bone is fixed in a vice and sections as thin as possible are cut with a fine saw, these are rubbed down with fine emery on a stone, and finally polished on a hone; they must be well washed to remove all debris and are better mounted dry, as they are apt to become too transparent when mounted in glycerine or other media.

Decalcifying Bone.

It is a very different matter to make sections of a bone after the earthy salts have been removed, as it can be cut, as easily as any other tissue, with the freezing microtome or razor.

To macerate small bones, such as mouse tail, half per cent. chromic acid will be sufficient; they should not be left in too long. Larger bones must be cut into small pieces and placed in half per cent. solution of chromic acid for a week or ten days, and then one-twentieth of the volume of hydrochloric acid added to the original fluid. First harden then soften them, five or six days after the addition of the acid will be enough according to the size of the bones. They should then be thoroughly washed in water for several days or a week,

according to size, to get rid of the lime salts, and then preserved in spirit.

Bone may be macerated in a saturated solution of picric acid, but it does not act so well as the above. It must be kept saturated by the addition of fresh crystals.

Make longitudinal sections through the head of a long bone such as the femur of a small kitten, double stain with picro-carmine and logwood and mount in Canada balsam. Take a portion of the lower jaw of a very young kitten near the condyle and decalcify it. When prepared cut transverse sections and double stain them with picro-carmine and logwood and mount in Canada balsam. Sections also through the carpus and tarsus of a fœtal child or kitten may be made and stained in the same manner.

The easiest specimens of growing bone to be got are from kittens just born, they should be decalcified in chromic acid as mentioned before.

MUSCULAR TISSUE.

Non-striped, Striped, Heart Muscle.

1. *Non-striped Muscle.* Make a preparation of the mesentery of newt or salamander in chromate of ammonia, page 13, and mount in glycerine. This specimen shows better when double stained with picro-carmine and logwood, page 36.

Examine first with a low power and see the distribution of the muscle fibres through the mesentery, and then with a high power to see the structure of the in-

dividual fibres; with the one-sixth the network in the nucleus can be seen but it will require a one-eighth or Zeiss' E to make out the fibrils passing out of the ends of the nucleus into the body of the cell, look carefully also for the transverse markings on the cell and the difference of its diameter in some places, compare their appearance with the plate in the Atlas of histology.

These fibres are very large and give a good idea of the structure of a non-striped muscle fibre.

In the large area in which this tissue is distributed throughout the body of a mammalian animal, the individual cells are very much smaller and their structure cannot be made out without a high power, especially as they lie very thickly together. In the intestine, however, a thin section will show a few fibres running up from the muscularis mucosæ to the basement membrane, and in these when well prepared and stained the same structures can be seen as in the much larger ones, in the mesentery of newt.

To see the intercellular substance by which the muscle fibres are held together, take a small portion cut longitudinally from the intestine and prepare it in chromate of ammonia, page 13, and stain in logwood. Cut longitudinal sections so that the circular muscle coat of the intestine is cut transversely, and it will be seen that each fibre is separated from the others by a homogeneous substance—the intercellular cement. There will be only a few nuclei cut through and these will be seen deeply stained.

2. *Striped Muscle.* Striped muscle is best shown

in one of the large water beetles, Hydrophylus piceus. There is another water beetle often sold for this purpose which does not answer so well, the Dytiscus marginalis, it may be distinguished by being smaller and having a yellow line round the margin of the upper surface.

Fresh Preparation of Muscle to show the Sarcolemma. Kill the beetle and remove one of the legs, open the chitinous covering and snip off a bit of the muscle, place it on a slide in a drop of distilled water, tease it out, cover and examine. The sarcolemma will be seen in places raised from the muscle substance.

Irrigate the same preparation with dilute acetic acid and the nuclei of the muscle corpuscles will soon come into view.

To make a permanent preparation stain a small portion of the leg muscle with logwood, tease it out carefully into as fine fibrils as possible in a drop of glycerine on a slide, cover and seal up with Hollis' glue. Dissect out some of the muscles of the thorax and mount in the same way. Examine the muscles in a transverse section of the tongue of any animal prepared in chromic acid mixture and double stained with picro-carmine and logwood.

3. *Heart Muscle.* Sections made from the heart of any small mammal will show the peculiarities of this variety of muscle fibre. Make transverse and longitudinal sections, and stain them with logwood. Note the position of the muscle corpuscles in the transverse sections, and the anastomosing of the fibres in those cut longitudinally.

NERVOUS STRUCTURES.

Medullated Nerve Fibres.

Dissect out the sciatic nerve of a frog and stain for a few minutes in half per cent. solution of nitrate of silver, wash well and expose to the light in distilled water until it has become of a brown colour; cut small portions and gently tease out so as to separate the fibrils, this must be done carefully in a drop of glycerine on a slide; cover and examine.

Nodes of Ranvier.

These will be found marked by black crosses in the course of the nerve fibre, the silver being heavily deposited at the node and penetrating a short distance either way giving the appearance of a cross. These nodes can be seen in the fresh nerve or in nerve hardened and stained in the ordinary manner.

Hardening Nerves.

Take any of the larger nerves from the animals used for material and harden some in 2 per cent. bichromate of potash, page 13; others in chromic acid mixture. Cut longitudinal and transverse sections of each of these and stain with logwood, mount in Canada balsam.

Non-Medullated Nerve Fibre.

This is best seen in a gold preparation of the tadpole's tail; it will require a high power to trace the finest fibres, Zeiss' E or F, but the one-twelfth oil immersion of Powell and Lealand with the binocular will enable the student to form a more correct idea of the structures he is looking at and their relations to one another.

Spinal Cord.

Procure a fresh spinal cord of calf from the butcher and cut it into pieces corresponding to the different regions. Prepare one half of each of these pieces in a 2 per cent. solution of bichromate of potash, page 13, and the other half in chromic acid mixture; those prepared in bichromate of potash may be put in as they are, but those in chromic acid mixture must be cut into lengths of about half an inch. The cord must be carefully handled care being taken not to squeeze it, and it should be cut with a sharp razor. Transverse sections of these must then be cut and stained with logwood, some may be stained with anilin blue, page 29, others double stained with carmine and indigo-carmine, page 37.

Sections can be cut by the microtome, but the razor must be very sharp and the cord well hardened. Very thin sections can also be cut by the hand and each section should be washed off the razor into spirit as it is cut. This should not be done with a brush but by dipping the razor into spirit. The cord prepared in bichromate of potash will show the nerve structures, that prepared in chromic acid mixture the neuroglia.

To shew the Large Multipolar Nerve Cells in the Spinal Cord.

Cut a piece of spinal cord through the middle longitudinally, take one side and holding it in the hand cut out with a sharp razor as thin a slice as possible of the anterior column, stain this deeply in picro-carmine. Take a small portion and place it in a drop of glycerine on a slide and tease out very carefully with two needles, this must be done gently and continued for some time until the whole piece is reduced to very minute portions, hardly discernible except by their colour; cover and seal up.

If this process has been carefully done, the large cells will be seen stained with picro-carmine and completely isolated with processes of different lengths, according to the care which has been exercised in teasing.

Several sections of the anterior column should be made and after staining they should be placed on a slide and examined with a low power; those which contain a large number of multipolar cells, will be readily seen.

Brain.

Small brains, such as rabbit's, harden very well whole by the spirit process, page 13, they must then be placed in absolute alcohol, before doing this the different parts should be separated, viz: frontal lobes, cerebellum, pons, &c.

After remaining in the alcohol a few days, sections may be cut of any of the parts to be examined and

stained with logwood, which shows up the structural elements better than any other stain.

Human brain is best hardened in 2 per cent. solution of bichromate of potash, page 13; it must be well washed until no more color comes away, before sections are cut. This method is equally applicable to all large brains.

Large sections of brain may be cut with the microtome, but for Histological work small sections will be found to show all that is required. It is a comparatively easy matter to cut a large section, but to pass this section through all the different re-agents and finally to get it laid out smoothly on the slide, without tearing, is a very difficult matter indeed.

Bichromate of ammonia (p. 13), may be used in the same manner as bichromate of potash, for all nervous structures.

To show the neuroglia of the brain, it must be cut in small pieces and hardened in the ordinary chromic acid mixture.

Pacinian Corpuscles.

These are best seen in the meso-rectum of cat, where they are visible to the naked eye as oval bead-like bodies. Cut out a portion of the meso-rectum and spread it on a flat piece of cork, fasten it at the sides by a few pins, and invert the cork in a vessel containing a 2 per cent. solution of bichromate of potash; let it remain in this for a few days, then cut it into small pieces and wash well, place them in a very dilute solution of logwood, one drop to a watch glass of distilled water will

be enough. They must be allowed to stain very gradually, as the logwood takes some time to penetrate the capsules. The solution of logwood must be changed several times as it is apt to become granular. It will take about 48 hours or longer, to stain the corpuscles thoroughly. When they have taken in the colour sufficiently, wash them well in plain water and mount in glycerine.

Vertical sections should also be made of the pad of a cat's foot, hardened in chromic acid mixture and stained in logwood.

BLOOD VESSELS.

Capillaries.

Take the tail of a half-grown Tadpole or common frog, and place it in a 5 per cent. solution of chromate of ammonium for 24 hours to remove the epithelium, then wash well until no colour comes away in the water, and double stain with carmine and indigo-carmine (page 37). Mount in Canada balsam.

By this process the capillaries will be deeply stained with carmine, and can be seen in their natural condition.

Examine the capillaries, in a gold preparation, of a Tadpole's tail stained with logwood.

Either of these preparations will show them in process of development from branched connective corpuscles. Examine them carefully for the nuclei of the walls, and observe in many the contained blood corpuscles.

Arteries and Veins.

Take the aorta of the dog or cat and prepare either in chromic acid mixture or spirit mixture (page 11). Make longitudinal and transverse sections, stain some with logwood and double stain others with picro-carmine and logwood (page 36). Mount in Canada balsam. Also make transverse sections of the whole aorta of rabbit and of smaller arteries and veins of the same and other animals, prepared as above. Stain with logwood and mount in Canada balsam. Examine these carefully to see the different amount of elastic tissue and non-striped muscle in the different arteries.

Veins are prepared in the same manner as arteries, but as they are so much slighter in structure it is easier to examine them *in situ* in such sections as tongue, kidney, &c.

Endothelium of Blood Vessels.

To examine the endothelium of a blood vessel it should be opened and then placed for two or three minutes in half per cent. solution of nitrate of silver, and exposed to the light in distilled water until it has become a brown colour. Then pin it out on a cork and tear off with the fine pointed forceps thin strips of the intima. Mount these in glycerine.

To examine the different coats of a blood vessel separately, it must be macerated for a few days in a 2 per cent. solution of bichromate of potash, well washed, and strips torn off from the different coats; these can be then stained and mounted.

Lymphatic Glands.

The lymphatic glands of the cat are very good for examination, and should be perfectly fresh; they are best hardened in chromic acid mixture.

Thin sections should be made with the microtome and stained with logwood. After washing, some of the thinnest should be placed with some water in a test tube and shaken for half an hour or more, to detach the corpuscles from the adenoid reticulum. They must be shaken steadily or they will be knocked to pieces. Two or three sections only should be shaken at one time. They are afterwards mounted in the usual manner in Canada balsam.

It is a good plan to inject a solution of Berlin blue into the lymph channels of a lymphatic gland, to demonstrate the passage of lymph through it. It is done by inserting the point of a hypodermic syringe, filled with a solution of Berlin blue, through the capsule of a fresh gland, and slowly injecting the colouring matter; the gland is then prepared in the usual manner, and sections cut and stained with logwood.

Thyroid Gland.

This may be prepared in precisely the same manner as a lymphatic gland.

SALIVARY GLANDS—PANCREAS.

May be hardened in chromic acid mixture or in spirit mixture and care must be taken that they are fresh and not over hardened. Sections may be cut by the microtome or by hand and stained in logwood.

TEETH.

Sections of hard teeth are prepared in the same way as bone. Teeth may be decalcified by the same process as that used for bone, page 71. Good sections of teeth *in situ* may be made by removing the lower jaw of some small animal as rat or mole and decalcifying it, after the lime salts have been thoroughly washed out, it should be soaked in gum for 24 hours and then cut with the freezing microtome.

Sections may either be mounted without staining in Canada balsam or they may be double stained with picro-carmine and logwood.

ALIMENTARY CANAL.

Stomach.

The stomach may be prepared in several different ways.

1*st Method.* Remove and open a fresh stomach of dog, cat or rabbit, and wash it slightly in dilute chromic acid, then place it in the ordinary chromic acid mixture and proceed as described at page 11.

2*nd Method.* To show the peptic cells take fresh stomach and wash quickly, then plunge into pure methylated spirit.

3*rd Method.* Put the stomach in Muller's fluid unwashed, for 48 hours. Then cut narrow strips of the mucous membrane about half an inch long by one eighth of an inch wide and wash these in one-tenth per cent. osmic acid, then place them in half per cent.

osmic acid from one to two hours to stain, then place them in one-sixth per cent. chromic acid and complete the hardening in the usual manner. The one-sixth per cent. chromic acid is here used without the addition of methylated spirit.

Some sections of stomach should be taken from the different parts, pylorus, cardiac end, &c., and these should be stained in logwood; those which are required to show the peptic cells should be stained in anilin blue.

Pyloric end of stomach with commencement of duodenum should be hardened in chromic acid mixture or if the whole stomach has been hardened a portion showing the junction of these two parts should be cut with the microtome and double-stained, a few sections also should be stained with logwood. Examine these sections for the gradual change in the epithelium as the one organ passes into the other.

Duodenum

May be hardened in chromic acid mixture and sections stained with logwood.

Notice Brunner glands cut in different sections, also goblet cells amongst the columnar epithelium and the fine non-striped muscle fibres running up from the muscularis mucosæ to the basement membrane.

Ileum.

The whole of the intestine may be hardened in chromic acid mixture or in spirit mixture, page 11, it must not be much handled and should be first slightly washed in very weak solution of chromic acid. Sec-

tions are best cut with the freezing microtome and may be stained in a great many different ways.

In a section containing a portion of Peyer's glands, the treble staining process, page 39, may be used, and the result will be very good as the Peyer's glands take on the green alone, without combining another colour with it, as all the other elements in this specimen do, so that they are brought out as brilliant light green bodies.

Ilio-Cœcal Valve.

A section should be made through the ilio-cœcal valve with a little of the intestine on either side of it, and this should be trebly stained by the process mentioned at page 39. Some sections should, however, always be stained with logwood to compare with the others, as although double and treble staining differentiate the various tissues, logwood brings out the structural element better than any other stain.

Solitary Glands.

Sections should be made through a piece of large intestine containing a solitary gland and this will be well brought out by the treble staining process.

LIVER.

The liver may be prepared for examination in three ways :—

1. By the ordinary chromic acid mixture, page 11.
2. By dilute spirit, page 13.
3. By Muller's fluid, page 12.

1. *The Chromic Acid method.* The liver must be perfectly fresh and cut into small pieces about half an inch square, these should be placed at once in the fluid without washing. A large quantity of blood will exude from them after being in the hardening fluid a short time, and it will be necessary to change it in many cases at the end of 12 hours. It is a good plan to shake the bottle containing the specimen in process of hardening occasionally, this must be done gently, so as to just alter their position, and when there is a quantity of sediment at the bottom of the bottle, and the fluid has lost its yellow color and begins to look muddy, it is time to change it. Portions of liver require changing a little oftener at first than other normal structures.

2. The *spirit method* is used in the ordinary manner for liver, but the dilute spirit will generally require changing once before using the pure methylated spirit.

3. *Muller's Fluid.* This may be used when large portions of the organ are to be hardened and when time is no object.

When the material is well hardened by either of these processes, beautiful sections may be cut with the freezing microtome, and they show best when stained with logwood.

The intra-cellular and intra-nuclear network is seen very well in the cells of the liver, and makes an interesting object for a moderately high power. The specimens should be searched for bile ducts cut transversely, looking like minute triangular openings between the cells.

LUNG

May be hardened in either chromic acid and spirit, or Muller's fluid, but to harden it well the fluid must be injected into the lung through the trachea. This is very easily done: the lung having been removed with a portion of the trachea attached, an ordinary brass syringe with ivory nozzle is filled with the hardening fluid, the nozzle inserted in the trachea, and the lungs gently distended with the fluid; when sufficiently full, the trachea is tied and a weight attached. The lungs are then placed in a tall vessel containing the hardening fluid, which is changed as often as necessary.

To show the Epithelium of the Alveoli

Inject through the trachea a $\frac{1}{8}$ per cent. solution of nitrate of silver, and then harden the lung by the spirit process (page 13), and make horizontal sections, these must be rather thick to get a correct idea of the epithelium as lining a cavity. These specimens will show the stomata between the epithelial cells.

Lung is best stained with logwood. Sections should be made through a bronchus and the small masses of ganglionic cells examined, these same sections may also be double or treble stained to differentiate the glands of the bronchi.

KIDNEY.

This organ is very well hardened in chromic acid mixture.

Remove the kidneys from a freshly killed animal,

take one and divide it transversely into several pieces and place them in the fluid. The other may be divided longitudinally by one cut, and large kidneys may be hardened in chromic acid in this way. Sections may be cut by the freezing microtome, and are best stained in logwood, or double stained in carmine and indigo-carmine (page 37). To show the minute structure of the cells in the collecting tubes, fine striation of epithelium, &c., Heidenhain's method is the best.

Cut the kidney into small pieces longitudinally in the direction of the pyramids, and place them in a 5 per cent. solution of chromate of ammonia, from 24—48 hours in a stoppered bottle, then wash for several hours until no more color comes away, changing the water several times, and place in dilute and then in strong spirit in the ordinary way (page 13).

Sections should be made both vertical and transverse of the cervical and medullary portion of the kidney, as well as large sections longitudinal and transverse of the whole organ; by this mean the general structure of the organ and the arrangement of the tubes can be examined, while in the smaller sections, some of which should be mounted under thin covers, the minute structure can be studied; for this no stain succeeds so well as logwood.

Examine sections of the cortical part for the striation of the epithelial cells, and notice the imbrication in some parts.

Bladder—Ureter.

Cut into small pieces and harden in chromic acid mixture.

To show Ganglia of Bladder.

Remove the bladder, empty it, and place in $\frac{1}{2}$ per cent. solution of gold chloride 2 hours in the dark. Then place it in water well acidulated with acetic acid, until it has become swollen up to a good size, then preserve it in glycerine.

The gold will be seen deposited in small patches here and there on the surface, one of these is removed with a pair of curved scissors and mounted in glycerine.

GENITAL ORGANS—MALE.

Testis.

The best hardening fluid for the testis is undoubtedly the chromic acid mixture. The organ is cut into small pieces, or deep cuts made into it with a sharp razor, according to the size. It must not be washed and should be handled as little as possible.

Sections are made in different directions through the various parts, and it is advisable to make some large sections through the corpus Highmori and globus major to show the structure and relation of the different parts; some of these sections will show the epididymis as well. These sections if at all large are very difficult to mount, as they break to pieces on the lifter, and it is often impossible to get them on to the slide whole; in this case the best plan is to use the cover glass as a lifter, and clean it afterwards, when the Canada balsam has set.

Sections of testis are best stained in logwood, or double stained in picro-carmine and logwood (page 36).

Some of the thinnest sections should be mounted under ·003 cover glasses, as the developing spermatozoa form very interesting objects for the highest powers.

Epididymis and Vas Deferens

Are hardened in the same way as testis, and epididymis is generally hardened and cut with the testis.

Stain with logwood.

Examine the columnar epithelial cells of the epididymis with very long ciliary processes. With a good object glass of moderate power these processes can be seen to be continuous with the longitudinal striation in the body of the cell, and can be traced by careful focussing through the striated line at the margin of the cells.

Prostate, Glans, Etc.

These can all be hardened in chromic acid mixture in the usual manner. The glans may be placed in gold chloride for two hours and then hardened in spirit, after which longitudinal sections will show the nerve structures. The glans of a small animal hardened in this way and cut longitudinally, will show the difference between the epithelium of the mucous surface and that in the meatus and commencement of the urethra; it will also show large bundles of medullated nerves arranged in a peculiar manner, and many other things worth studying.

GENITAL ORGANS—FEMALE.

Uterus, Fallopian Tubes, Vagina.

Can all be hardened in chromic acid mixture, and should not be washed unless absolutely necessary.

To examine the glands of the uterus it is better to use an animal that has borne young.

These organs may also be hardened whole in Muller's fluid or bichromate of potash. The sections are best stained in logwood.

Ovary

Is best hardened in chromic acid mixture and should not be handled more than is absolutely necessary. The whole organ may also be hardened in Muller's fluid.

Mammary Gland.

Cut small pieces and place in chromic acid mixture, when hardened stain with logwood.

Placenta.

The placenta of guinea-pig is the best for examination, and should be taken a little after half the period of gestation has passed. It may be prepared in chromic acid mixture or in spirit mixture. Vertical sections should be cut as thin as possible, and stained with logwood.

SPERMATOZOA.

The living spermatozoa of Triton cristatus make a most beautiful preparation and are readily procured.

Take a large male newt which may be known by the serrated crest or fin along the back, and kill it, quickly remove the viscera, and the testes will be found, generally two or three each side, as small round bodies which cannot easily be mistaken. Take one of these and make a small cut in it, remove some of the milky fluid which exudes to a slide, and add a little salt solution or distilled water. Cover and examine. A power about $\frac{1}{8}$ will be required to see the spiral filament well.

A large number of spermatozoa will be seen in the field making slight lashing movements with the long filiform body, and on closer examination the filament will be seen in rapid movement; this movement commences at the elliptical body at the base of the head, and gives the idea at first sight that the filament is being poured out from it. After watching for some little time, the movement will become slower, and it can then be seen that the filament is attached to the body by a membrane, and that it is waved rapidly from side to side, by carefully watching it as the motion gets slower and nearly stops the membrane connecting the filament to the body, can be clearly seen.

To make a Permanent Preparation of Newt's Spermatozoa.

Place the testes in 5 per cent. solution of chromate of ammonia for 24 hours; wash until no colour comes away in distilled water, then divide one of the testes in two, and taking one half in a pair of forceps press the cut surface on a glass slide, a small quantity of

fluid will adhere to the slide, to this add a small drop of glycerine and gently mix the two fluids. Cover and examine.

To Stain Newt's Spermatozoa.

After having washed away all traces of the chromate of ammonia, make an incision into the testis nearly dividing it, and place it in undiluted logwood stain for an hour or more, then wash away all superfluous stain and mount in glycerine. Spermatozoa may be double stained, but it is a very tedious process, as a little too long immersion in either stain spoils the whole process. The best way is, after washing off the superfluous logwood stain, to dip a portion of the testis in undiluted spirituous solution of rosein ; a little must be mounted to see if the stain is deep enough, if not it must be dipped again.

It is possible in this way to get spermatozoa of newt or salamander with the long pointed head stained with rosein, while all the other parts are stained with logwood.

Mammalian Spermatozoa.

To make preparations of mammalian spermatozoa, a little glycerine is placed in a watch-glass, and one or two drops of absolute alcohol added. A cut is then made into the globus major of a fresh testicle, and a little of the fluid removed on the point of a knife and placed on a slide ; a small drop of the glycerine is then mixed with it, it is then covered and sealed with Hollis' glue.

Human Spermatozoa.

A small drop of semen is mixed with glycerine, to which a little absolute alcohol has been added; it is then covered and sealed up. It is best in this case to use thin covers that have been measured, and to note their thickness on the label, as the human spermatozoon is so very minute it requires the highest powers to make out the filament.

SPECIAL SENSES.

Internal Ear. Cochlea.

The guinea-pig is the best animal, as the large tympanic bulla is easily exposed. Remove the periosteum from the bulla, and open it carefully with the point of a pair of straight scissors; as soon as a small opening has been made it can be enlarged, and the cochlea will be at once seen: as much of the surrounding bone must be removed as can be done without injury to the parts required, and it will be ready for preparation.

This may be done in several ways, but the two following are the best.

1. Place it at once in absolute alcohol, and let it remain 24-48 hours, then place it in $\frac{1}{10}$ per cent. solution of osmic acid for 24 hours. After this place it in a half per cent. solution of chromic acid, to which 1-2 drops of hydrochloric acid have been added. Let it remain in this until the bone is softened throughout.

2. Place the piece of bone in the ordinary chromic acid mixture for a week, and then remove it to $\frac{1}{2}$ per cent. chromic acid, to which 1-2 drops of hydrochloric

acid have been added. Remove when the bone is softened.

When the bone is decalcified by either of these processes, it must be washed for several days, to remove the lime salt. It is then ready for cutting sections. In the case of the internal ear, sections are much better cut by hand, as the fine structures such as the rods of Corti are quite disarranged by freezing, and cutting with a microtome.

The bone must now be placed in gum solution, for 24 hours, and then removed to spirit slightly diluted with water. If the spirit is too strong, the gum will form a substance like chalk, and quite as hard; this can, however, be softened by placing it in water. When the gum is sufficiently hardened by the spirit, it can be embedded in wax mass, and sections cut by hand in the ordinary manner. These sections must be very gently handled, and should not be lifted with a needle but with a fine camel hair pencil. They are best stained in logwood. It is a good plan to stain the whole bone in logwood before placing it in the gum solution, but care must be taken that it is not too deeply stained. The undiluted logwood should be used, and it will require about 6 hours or more to stain it thoroughly.

Sections must be cut through the semi-circular canals and also through the cochlea, and the cochlea should be so embedded that sections will be cut through its whole length.

NASAL ORGAN.

The nasal organ is prepared in the same manner as the internal ear, and can be conveniently removed and hardened with it. Transverse sections should be made through the anterior part to show the membrane of the respiratory part and transverse sections further back, and show the olfactory membrane with its peculiar epithelium.

The septum of the nose should also be carefully removed from a specimen, and longitudinal sections made of it: if these sections are very carefully handled, they will show both the respiratory and olfactory epithelium very well. They are best stained in logwood and mounted in Canada balsam.

EYE.

The eye may be hardened in chromic acid mixture or in Muller's fluid. It must be removed without squeezing, and a few incisions made, it can then be hardened whole. When sufficiently hard, divide the eye longitudinally with a sharp razor.

Retina.

The retina will be found lying on the inside of the posterior part of the eye, from which it may be gently detached by a spear-shaped knife, it may be then frozen and sections cut; it is better, however, to stain it first, as thin sections are so transparent it is difficult to see them. When prepared in Muller's fluid the retina is

very brittle, but in chromic acid mixture it is much tougher and can then be cut in strips and several frozen together in the microtome. Muller's fluid shows the nervous structure best, chromic acid the connective tissue. Good sections of the retina may be obtained by cutting the whole eye of the frog, and it may be double stained first.

To do this, first place the whole eye in a strong solution of rosein until it is deeply stained, then wash away the superfluous stain in methylated spirit, next place the eye for a short time in strong solution of iodine green and wash it well, soak in gum solution and freeze. By this means very good sections can be obtained, the granular layers having stained with the iodine green, the others with rosein.

They must be mounted in Canada balsam and should not be left long in spirit.

Cornea.

The cornea may be removed from an eye hardened either in chromic acid mixture or in Muller's fluid, and sections made with the freezing microtome; they are best stained with logwood.

To demonstrate the Corneal Corpuscles and Nerves.

Remove the cornea from an animal just killed: this is done by cutting round the margin with fine curved scissors. Place the cornea in $\frac{1}{2}$ per cent. solution of gold chloride in the dark. Let it remain for from one hour to one hour and a half for a guinea-pig, an hour

and a half to two hours for a rabbit. Then place it in distilled water, which must be changed once or twice, for 24-36 hours exposed to the light, it will then have become a violet colour.

It is now placed in a mixture consisting of

Pure glycerine, 1 part.
Distilled water, 2 parts.

Let it remain in this for two or three days in the dark. It is then taken out, and gently washed and placed in a wide mouthed vessel containing a filtered nearly saturated solution of tartaric acid. As it absorbs this liquid, the colour will become darker and it will sink to the bottom of the vessel. The vessel is now plunged into water at a temperature of 40° to 50° C. to such a depth that the two fluids will stand at the same height.

Sections may now be made with a very sharp razor by holding the cornea between the finger and thumb of the left hand. This requires great care, and cannot be done without a good deal of practice. The sections are mounted in glycerine and sealed with Hollis' glue.

Before cutting the sections, while the cornea is still in the distilled water, it is well to pass a camel's hair pencil gently over the surface, to remove the gold deposited there.

Iris and Sclerotic.

A portion of the eye containing part of the cornea sclerotic and iris may be cut out, and frozen. Sections of this stained with logwood, show the junction of the cornea and sclerotic, and the structure of the iris.

Lens.

In cutting sections of the whole eye of frog, good sections of the lens can be obtained. In the whole eye prepared by either of the methods given, the lens will be found hardened, when the eye is opened; and sections may be cut by the freezing microtome, but they generally break up if thin; enough, however, can be obtained to enable the student to examine into the structure.

PRACTICAL PATHOLOGY.

On Preparing and Mounting Pathological Specimens.

Very little need be said about preparing pathological specimens, as most of the processes already mentioned will apply equally well to morbid tissues. It is often necessary to find out at once what a tumour or new growth consists of, and for this purpose it is necessary to examine the fresh tissue. In some cases this is sufficiently hard to be frozen and sections made, while in others a small portion can be teased out on a slide and examined.

This may give a rough idea of what the morbid growth is composed, but for a thorough examination and when it is desired to keep preparations of any disease, a complete process of hardening must be adopted, and sections cut and stained as in normal tissues, and for this purpose it is necessary to have the material as fresh as possible.

To make Permanent Preparations of a Cancer in a Short Time.

This method may be applied to most of the sarcomata and carcinomata, and is valuable, as it can be used on portions of morbid tissue excised from the living body. Snip off a small portion of any morbid growth, such as cancer, with a pair of curved scissors.

Place it in a mixture of dilute spirit (page 13) for 12 hours, then remove it to pure methylated spirit for 12 hours and finally to absolute alcohol for 12 hours. It will then in all probability be fit to cut sections from. Imbed in wax mass and cut some sections by hand, stain with logwood and mount in Canada balsam.

There are some morbid growths, such as medullary carcinoma, which cannot be dealt with in this way, and they must be hardened in the chromic acid mixture in the usual manner.

In all morbid growths where there is a large epithelial element, they can be best hardened in spirit mixture. When large masses are to be hardened, Muller's fluid or 2 per cent. bichromate of potash are necessary, and must be used in the manner described for normal tissue at page 12.

It is quite as important that pathological specimens should be properly hardened as normal tissues, but how seldom is this done. In the first place it is difficult to get the morbid tissues fresh enough, and yet they are often put on one side or at most placed in the lump in a very small quantity of methylated spirit for some time before being hardened, and it is expected that good sections can then be prepared from them.

Nothing is more erroneous than this idea; the subject has been probably dead 24 hours at the least when the post mortem is made, often longer, and in summer especially, this means utter ruin to such organs as the spleen. How important is it therefore that such organs as are fresh should be put in the hardening medium at once. For this purpose a wide-mouthed bottle of chro-

mic acid mixture should be taken to every post mortem examination, and small bits of any organ that may seem interesting on any account may be put in. A small paper label may be tied on and they can be separated afterwards.

Almost every morbid growth can be hardened in the chromic acid mixture (page 11), in the same manner as normal tissues. Brain and spinal cord, however, are better prepared in 2 per cent. solution of bichromate of potash (page 13).

On Double and Treble Staining Morbid Growths.

Some very good results will be obtained if the different staining processes mentioned, as well as any others that may suggest themselves, are tried on different morbid growths.

For instance, well hardened sections of rodent ulcer and epithelioma may be stained by the indigo-carmine process (page 37) and carefully compared. Other sections of the same material should be then stained with rosein and iodine green (see treble staining) and again compared. In this way, some definite result may be worked out, which by using other specimens of the same disease may be confirmed.

Large Sections of Pathological Specimens.

If a large section is wanted of any morbid growth to show the distinction between the healthy and diseased parts, such as a section through a cancer and the side of the uterus from which it is growing, it is better to

cut a moderately thick slice, say about a quarter of an inch thick, and harden it in chromic acid mixture, than to harden the whole mass in spirit, as it will be found a very difficult matter to cut large sections of spirit hardened material; they become so hard that they make the knife jump, and the section is consequently uneven.

AMYLOID DEGENERATION

Is best hardened in chromic acid mixture, and in kidney especially is very well shown by this method. To show the amyloid substance well it must be stained a different colour to the surrounding tissue, and this may be done by several of the double staining processes; as the anilin dyes seem to have a special affinity for amyloid degeneration, the following should be tried:

Carmine and indigo carmine (page 37).
Rosein and iodine green.
Eosin and anilin blue.

and many other combinations.

HYDATIDS.

To make a preparation of hydatid cysts, take a portion of the wall of a large cyst and scrape off some of the gelatinous matter adhering to it. Place a little of this on a slide and tease it gently in a drop of glycerine, cover and examine. If there are any small cysts showing hooklets, &c., well, seal it up with Hollis' glue.

SHORT HISTORY OF THE MANNER IN WHICH A PORTION OF MORBID GROWTH IS PREPARED BY THE CHROMIC ACID METHOD.

1st day. Small pieces placed in chromic acid mixture (page 11).

2nd day. Fluid changed.

5th day. Fluid changed.

8th day. Fluid changed.

9th day. Spirit mixture (page 13).

10th day. Pure methylated spirit.

14th day. Plain water.

15th day. Mucilage.

16th day. Section cut, stained, and mounted.

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MEMORANDA AND FORMULÆ.